aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Animal and Veterinary Advances 5 (6): 402-409, 2010
ISSN 1683-9919

A Novel 15 bp Deletion Mutation at *KAP16.5* Locus in Cashmere Goat of China

[1]Liu Wu Jun, [1]Fang Yi, [1]Shao Yong-gang, [2]Fang Guang Xin,
[6]Tian Ke Chuan, [1]Huang Xi Xia, [4,5]Chen Hong and [3]Zhang Fu Quan
[1]College of Animal Science, Xinjiang Agricultural University,
Urumqi, Xinjiang, 830052, People's Republic of China
[2]The Foreign Funds Project Office of Animal Husbandry Bureau,
Urumqi, Xinjiang, 830001, People's Republic of China
[3]Goat Research Center of Aksu Prefecture, Aksu City, Xinjiang,
843000, People's Republic of China
[4]College of Animal Science and Technology, Northwest A and F University,
Shaanxi Key Laboratory of Molecular Biology for Agriculture,
Yangling, Shaanxi 712100, People's Republic of China
[5]Institute of Cellular and Molecular Biology, Xuzhou Normal University,
Xuzhou, Jiangsu 221116, People's Republic of China
[6]Xinjiang Academy of Animal Science, Urumqi, Xinjiang 830000,
People's Republic of China

Abstract: In this study, a novel deletion mutation in the whole CDS region at cashmere goat *KAP16.5* gene locus is reported, which result showed the deletion of *KAP16.5* gene in 816 cashmere goat samples was firstly detected in China. At the same time, parts of these samples sequenced. However, there are not many studies of goat *KAP16.5* gene and its polymorphisms in literary sources. The polymorphism was studied in three Xinjiang local goat breeds of China. The results showed that there was 15 bp-deletion mutation in this gene. The frequencies of the *KAP16.5*-Y allele in Xinjiang goat (n = 220), Nanjiang cashmere goat (n = 310) and BoGeDa cashmere goat breeds (n = 286) were 0.9591, 0.8694 and 0.8846, respectively. The χ^2-test showed that the genotype distributions in these three cashmere goat breeds were not in agreement with Hardy-Weinberg equilibrium. According to the classification of PIC, Nanjiang cashmere goat was more polymorphic at this locus. Moreover, the 15 bp-deletion mutation was described at *KAP16.5* locus for the first time and not have described at *KAP16.5* locus. Then, the deletion polymorphisms within the *KAP16.5* have been associated with economic production traits data in Nanjiang cashmere goat breed shown no significant ($p>0.05$).

Key words: Cashmere goat, goat, cashmere traits, breed, *KAP16.5* gene, deletion mutation

INTRODUCTION

The hair keratin-associated proteins are a major component of the matrix between the hair keratins form intermediate filaments and are thought to form the rigid hair shaft through

Corresponding Author: Zhang Fu Quan, Goat Research Center of Aksu Prefecture,
Aksu City, Xinjiang, 843000, People's Republic of China

a cross-linked network with the hair keratins form intermediate filaments (Powell and Rogers, 1994). Mammalian skin consists of three major compartments: epithelium, dermis and hypodermis. It is important that epidermis is a derivative of the surface ectoderm, as a protective barrier and specific appendages including hair, nails and different eccrine glands (Jonker *et al*., 2004; Bond *et al*., 1996). The cuticle, the cortex and the medulla is a main structure of all hairs and wool (Bond *et al*., 1996; Langbein *et al*., 1999, 2001; Hawkins and Ragnarsdóttir, 2009; Feughelman, 2002). The internal cortical cells are long polyhedral spindle-shaped structures (Jones, 2001), which mechanically, are the most important component of any α-keratin fiber (Feughelman, 2002). The flattened overlapping, cuticle cells surround the cortex and forms the external layer of the fiber (Marshall *et al*., 1991). The structure of the cashmere fiber largely involves the expression of hair keratins and their keratins-associated proteins (Langbein *et al*., 1999, 2001). The keratins and Keratin-Associated Proteins (KAPs) are a large heterogeneous group of proteins that make up about 90% of the wool fiber (Powell and Rogers, 1994). The human hair is very resistant to external stimuli and high stability is due to keratin (Barba *et al*., 2009). The keratins and Keratin-Associated Proteins (KAPs) plays an essential role in hairs and wool.

KAPs are encoded by a large number of multigene families. Furthermore, the KAP genes are small in size less than 1 kb, generally contain a single exon. The KAPs have been divided into three categories, the high sulphur KAPs (<30 mol% cysteine content), the ultra-high sulphur KAPs (>30 mol% cysteine content) and the high tyrosine/glycine KAPs (Barba *et al*., 2009). The hair keratins represent the type I (acidic) and type II (basic) two multigene families. They form the 8-10 nm intermediate filaments (KIF) of trichocytes by co-polymerization of type I and type II members, which are differentially expressed during hair fiber development (Langbein *et al*., 1999, 2001). Previous studies have introduced these KAPs are using the abbreviations KAP1.n through KAP23.n for these members known at that time with n referring to a number identifying individual members, also subdivided into 23 distinct families (Rogers and Powell, 1993) and more than 100 KAP genes have been isolated from human and other mammalian species. Genetic markers for the keratin and keratin-associated protein genes have been associated with variation in fiber diameter and staple strength (McLaren *et al*., 1997).

The *KAP16.5* gene is one of high sulphur KAPs, which are important for the hair structure. Apparent molecular weights of the high sulphur proteins were 26.5-43.0 kd, but it is probably higher than the real values 75-150% (Marshall, 1983). The high sulphur proteins predominantly found in the cuticle with some also found in the cortex (Irvine, 2005). All *KAP16* gene exhibit strong expression in a narrowly defined pattern restricted to the lower and middle cortical region of the hair shaft in both developing and cycling hair (Pruett *et al*., 2004).

To date, no polymorphisms of *KAP16.5* gene have been reported for China cashmere goat. Therefore, the aim of our study is analyzed the genetic variations of *KAP16.5* gene in 816 goat individual in China by electrophoresed on agarose gels, PAGE and DNA sequencing methods.

MATERIALS AND METHODS

Animal Source

Eight hundred and sixteen all unrelated animals (Xinjiang goat, n = 220; Nanjiang cashmere goat, n = 310; Bogeda cashmere goat, n = 286) were collected and used in this study in March to December of 2009. The Xinjiang goat were from the breeding centre of KuErLe of XinJiang in China, the Nanjiang cashmere goat were from AkeSu Goat Research Center of XinJiang and Bogeda cashmere goat were from Urumuqi of XinJiang in China. Many records of cashmere traits and body weight were used for statistical analysis.

3DNA Preparation and Primer Design

Genomic DNA of 816 cashmere goat were isolated from 2% heparin-treated blood samples and stored at -80°C, following standard procedures (Sambrook and Russell, 2001). According to the sequence of *KAP16.5* (GenBank accession number AY510117), one pair of PCR primers was designed with Primer5.0, as follow:

- Forward: 5' -AGCAAACCAAACTCACCACC- 3'
- Reverse: 5' -TTCAGATGTCGGAGTGGGAT- 3'

It was used to amplify 272 bp PCR products for cashmere goat *KAP16.5* gene of the whole CDS region.

PCR Amplification

One pair of PCR primer were designed using Primer 5.0 software to amplify the whole CDS region of Capra *KAP16.5* gene (high sulphur KAPs) (GenBank accession number AY510117), the size of the PCR products was 272 bp. The 25 μL volume contained: 50 ng genomic DNA, 0.5 μM of each primer, 1×Buffer (including 1.5 mM $MgCl_2$), 200 μM dNTPs and 0.625 units of Taq DNA polymerase (MBI). The PCR was performed using the following program: 94°C for 5min followed by 33 cycles of 94°C for 40 sec, annealing for 35 sec and 72°C for 35 sec and a final extension at 72°C for 10 min.

Deletion Mutation Polymorphism in the *KAP16.5* Gene and DNA Sequencing

PCR products were electrophoresed on 4% agarose gels with 1×TBE buffer (89 mM Tris, 89 mM boric acid and 2 mM Na_2EDTA), containing 200 ng mL^{-1} ethidium bromide. A 6.5 μL aliquot of PCR products was added to 1.5 μL of loading dye (0.025% bromophenol blue, 0.025% xylene cyanol, 40%, w/v sucrose) and the gels were run at a constant voltage (100 V) for 0.5-1.0 h.

Aliquots of 5 μL PCR products were loaded on 10% PAGE gel (80×73×0.75 mm) in 1×TBE buffer and constant voltage 110 V for 3.5 h. The gel was stained with 0.1% silver nitrate solution. The two methods show equal data results: genotype YY, YZ and ZZ.

The PCR fragments from different patterns in the three breeds were amplified by the pair of primer, then sequenced in both directions by ABI PRIZM 272 DNA sequencer (PerkinElmer) analyze the sequences with BioXM software (version 2.6).

Statistical Methods and Analysis

In these Chinese goat breeds, base on the genotypes of *KAP16.5* locus, genotypic frequencies, allelic frequencies and Hardy-Weinberg equilibriums were directly. Differences in genotypic frequencies at Capra hircus *KAP16.5* locus among Xinjiang indigenous goat and cashmere goat populations in China were analyzed using a χ^2-test, which were performed by SPSS software (1999). Population genetic indexes, such as He (gene heterozygosity), Ho (gene homozygosity), Ne (effective allele numbers) and PIC (Polymorphism Information Content) were calculated according to Nei and Roychoudhury (1974) and Nei and Li (1979), respectively.

$$H_o = \sum_{i=1}^{n} P_i^2;\ Ne = 1 \Big/ \sum_{i=1}^{n} P_i^2;\ PIC = 1 - \sum_{i=1}^{m} P_i^2 - \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} 2P_i^2 P_j^2$$

RESULTS

Few studies related to polymorphisms of *KAP16.5* gene in cashmere goat had been reported. In this study, the entire CDS region and its flanking region of cashmere *KAP16.5* gene demonstrated polymorphic patterns in three cashmere goat populations of China. However, the deletion polymorphisms (named genotype YY, YZ and ZZ) were firstly detected at *KAP16.5* locus (Fig. 1a, b).

Frequencies of allele *KAP16.5-Y* allele in the analyzed populations were 0.8694, 0.8846 and 0.9591 for Nanjang cashmere goat (NJG), BoGeDa cashmere goat (BGD) and Xinjiang goat (XJG), respectively. The genotypic frequencies at *KAP16.5* locus were significantly different among Xinjiang goat, Nanjiang cashmere goat and Bogeda cashmere goat based on a χ^2-test (χ^2 = 34.588, df = 4, $p<0.001$), as well as allelic frequencies among the three populations (χ^2 = 8.721, df = 2, $p<0.05$). The χ^2-test showed that the genotype distributions of NJG, BGD and XJG breeds were not in agreement with Hardy-Weinberg equilibrium (Table 3). In present populations, the population genetic parameters (namely, homozygosity, heterozygosity, effective allele numbers (Ne) and PIC (Polymorphism Information Content)) were calculated (Table 2). According to the classification of PIC (high polymorphism if PIC value>0.5, median polymorphism if 0.25<PIC value<0.5 and low polymorphism if PIC value<0.25) (Botstein *et al*., 1980). Genotypic and allele frequencies of *KAP16.5* gene in the three breeds were shown in Table 1 and 2. Value of homozygosty estimate varied from 0.773 (NJG) to 0.922 (XJG) and Ne ranged from 1.085 (XJG) to 1.294 (NJG). PIC values varied from 0.075 (XJG) to 0.201 (NJG). The three goat populations were at low polymorphic level.

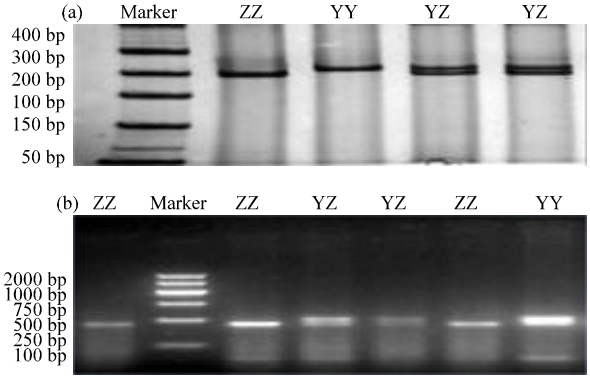

Fig. 1: (a) The 10% PAGE electrophoresis patterns of 272 bp PCR products of *KAP16.5* gene in goat (ZZ: 257 bp; YY: 272 bp). (b) The 4% agarose gels electrophoresis patterns of 272 bp PCR products of *KAP16.5* gene in goat (ZZ: 257 bp; YY: 272 bp)

Table 1: Genotype distribution and allelic frequencies at the *KAP16.5* gene locus

	Observed genotypes				Allelic frequencies		
Breeds	YY	YZ	ZZ	N	Z	Y	χ^2 (HWE)
XJG	205.0000	12.0000	3.0000	220	0.0409	0.9591	20.452
	0.9318	0.0546	0.0136				
NJG	239.0000	61.0000	10.0000	310	0.1306	0.8694	5.545
	0.7710	0.1968	0.0322				
BGDG	238.0000	30.0000	18.0000	286	0.1154	0.8846	67.598
	0.8322	0.1049	0.0629				

Genotype frequencies at the *KAP16.5* gene locus; χ^2 (HWE): Hardy-weinberg equilibrium χ^2 value; NJG: NanJiang cashmere goat; BGDG: BoGeDa cashmere goat; XJG: XinJiang cashmere goat

Table 2: Genetic index in three Xinjiang local goat in China

Breeds	Type	Ho	He	Ne	PIC
NJG	Cashmere	0.773	0.227	1.294	P = 0.201
XJG	Cashmere	0.922	0.078	1.085	P = 0.075
BGDG	Cashmere	0.796	0.204	1.257	P = 0.183

Ho: Gene homozygosities; He: Gene heterozygosities; Ne: Effective allele number; PIC: Polymorphic information content

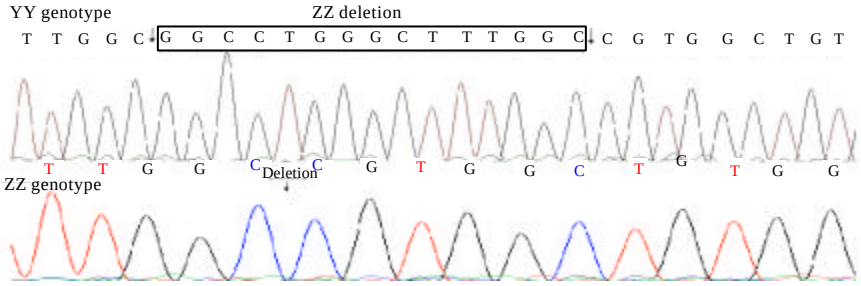

Fig. 2: The sequencing and sequence comparison results of *KAP16.5*gene in goat. The sequencing results of *KAP16.5* gene YY (up) and ZZ (down, Deletion) genotype in goat

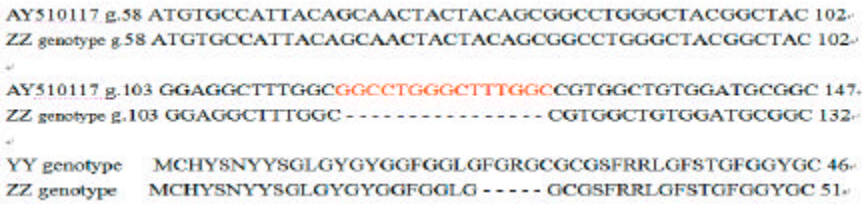

Fig. 3: The sequencing, sequence and Amino acid sequence comparison results of *KAP16.5* gene in goat. The sequencing and sequence comparison results of *KAP16.5* gene in goat. Amino acid sequence comparison of *KAP16.5* protein, the individuals of ZZ genotype delete 15 bp -GGCCTGGGCTTTGGC-

Taking nucleotide sequence AY510117 (GenBank Accession Number AY510117) as criterion, nucleotide sequences from 24 individuals with 3 different genotypes were aligned with the multiple sequence alignment program ClustalW (http://www.ebi.ac.uk/clustalw). A novel 15-bp deletion was detected among them (Fig. 2, 3; GenBank Accession No. AY510117). According to the mutation nomenclature and proposals (Den Dunnen and Antonarakis, 2000), the 15 bp deletion mutation was described as r.115-129 deletion (-GGCCTGGGCTTTGGC-) mutation (Fig. 2, 3). Observed significant differences of genotypic frequencies for YY, YZ and ZZ indicate that the breeds are different according to content of allele Z-with deletion by χ^2-test ($\chi^2 = 8.721$, df = 2, $p<0.05$) (Table 3). Taking in consideration, that the breeds studied represent different capara utility type (economic production of cashmere traits), the genotypic distribution possibly effect utility type of the breeds. Allele *KAP16.5* gene was detected in all the cashmere goat breeds. Probably, deletion was not spread in the breed discussed.

The association of novel 15 bp deletion in *KAP16.5* gene with cashmere production traits (body weight after combed, cashmere fiber diameter, down cashmere thickness,

Table 3: Association of genotypes at the *KAP16.5* gene with cashmere traits in NanJiang cashmere goat

	Genotypes at KAP13.1 gene			
	YY	YZ	ZZ	
Cashmere traits	(Mean±SE)			p-value
BWC (kg)	21.427±0.174	21.230±0.344	21.500±0.850	>0.05
CD (μm)	15.758±0.055	15.625±0.110	15.706±0.271	>0.05
DCT (mm)	4.667±0.045	4.695±0.090	5.100±0.221	>0.05
CY (g)	464.870±6.701	468.770±13.265	490.000±32.761	>0.05

BWC: Bodyweight after combed; CD: Cashmere fineness; DCT: Down cashmere thickness; CY: Cashmere yield

cashmere yield) in Nanjang cashmere goat were analyzed (Table 3). The cashmere production traits in the records had no significant associated with genotypes studied ($p>0.05$).

DISCUSSION

Many previous studies reported genetic polymorphisms in human high sulfur hair keratin-associated protein, which also play an important role in determining various wool traits as well as the variation in fiber diameter (McLaren *et al*., 1997; Parsons *et al*., 1994). Few studies have been reported related to *KAP16.5* gene in human and mouse, which also not have been reported in goat. It is known that the fiber diameter, cashmere yield and down cashmere thickness were important cashmere production traits in cashmere goat. Present study is considered as the firstly detected a deletion mutation in the coding region of *KAP16.5* gene in these cashmere goat breeds in China.

Notably, all *KAP16* gene exhibit strong expression in a narrowly defined pattern restricted to the lower and middle cortical region of the hair shaft in both developing and cycling hair. The study of 15-bp deletion mutation in our results was not a casual mutation, which is essential for *KAP16.5* gene CDS region, it showed 15-bp deletion mutation in *KAP16.5* protein Cys, Ala, Cys, Cys and Ala, which may be linked to cashmere goat in the coding or regulatory regions of the gene. The result of polymorphic level of Xinjiang goat population was lower than that of Nanjiang and Bogeda cashmere goat population. In order to better understand the detailed genetic variation of *KAP16.5* gene, we analyzed it with cashmere production traits in the records. Moreover, the research results revealed no significant impact for genotypes studied ($p>0.05$) by artificial selection and breeding. So, the objective of this study was to detect the polymorphism within goat *KAP16.5* gene in Chinese animals, which may possibly contributed to conducting association analysis and evaluating them as genetic markers in cashmere production and other performance for animal breeding and genetics. In order to get more genetic variation information we would do pay more attention to further research in the future.

The χ^2-test results obtained in present study that may be caused by nonrandom mating and among the three Xinjiang local goat breed selected some of the excellent individuals could violate the Hardy-Weinberg equilibrium.

For all the available information concerning *KAP16.5* gene in human and mouse, there is no information known for cashmere goat. Our study is considered as the firstly detected the deletion novel mutation in the coding region of *KAP16.5* gene in three cashmere goat breeds and associated with cashmere production traits of China. Therefore, the presence of 15 bp deletion in *KAP16.5* gene might straddling influence the body weight after combed, cashmere fiber diameter, down cashmere thickness, cashmere yield. The *KAP16.5* gene seems to be promising as it plays an important role in fiber diameter traits. It is important that cashmere production traits to the livestock industry, it appears clearly that essential further researches on *KAP16.5* gene in the livestock should be done.

CONCLUSION

In this study, we revealed a novel 15-bp deletion polymorphism in the whole CDS region of *KAP16.5* gene. The deletion mutation is not significantly associated with body weight after combed, cashmere fiber diameter, down cashmere thickness and cashmere yield trait. The Z allele was a straddling influence in the three goat breeds and it should be pay attention from the breeding schemes of goat. Therefore, this study considered as a preliminary important research for improving the Xinjiang local goat breeds and the breeding of genuine cashmere in China. Also, the present study is one of initial studies performed on China goat cashmere traits and provides preliminary information about alleles and genotypes of *KAP16.5* gene in three goat breeds of China.

ACKNOWLEDGMENTS

This study was supported by The project of key Science and Research Program of Xinjiang High School SandR Plan (No. 20090608115118343), GEF Applied Research Project (No. GEF052456 CHA). The project of National Key Technology RandD Program in the 11th Five-year Plan of China (2008BADB2B05).

REFERENCES

Barba, C., S. Méndez, M. Martí, J.L. Parra and L. Coderch, 2009. Water content of hair and nails. Thermochimica Acta, 494: 136-140.

Bond, J.J., P.C. Wynn and G.P.M. Moore, 1996. Effects of epidermal growth factor and transforming growth factor alpha on the function of wool follicles in culture. Arch. Dermatol. Res., 288: 373-382.

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

Den Dunnen, J.T. and S.E. Antonarakis, 2000. Mutation nomenclature extensions and suggestions to describe complex mutations: A discussion. Hum. Mutat., 15: 7-12.

Feughelman, M., 2002. Natural protein fibers. J. Applied Polymer Sci., 83: 489-507.

Hawkins, D.P. and K.V. Ragnarsdóttir, 2009. The Cu, Mn and Zn concentration of sheep wool: Influence of washing procedures, age and colour of matrix. Sci. Total Environ., 407: 4140-4148.

Irvine, A.D., 2005. Inherited defects in keratins. Clin. Dermatol., 23: 6-14.

Jones, L.N., 2001. Hair structure anatomy and comparative anatomy. Clin. Dermatol., 19: 95-103.

Jonker, L., K. Ralf, A.W. Andrew, W. Ilka and P. Heiko, 2004. Pax9 is required for filiform papilla development and suppresses skin-specific differentiation of the mammalian tongue epithelium. Mechanisms Dev., 121: 1313-1322.

Langbein, L., M.A. Rogers, H. Winter, S. Praetzel, U. Beckhaus, H.R. Rackwitz and J. Schweizer, 1999. The catalog of human hair keratins. I. Expression of the nine type 1 members in the hair follicle. J. Biol. Chem., 274: 19874-19884.

Langbein, L., M.A. Rogers, H. Winter, S. Praetzel and J. Schweizer, 2001. The catalog of human hair keratins-II. Expression of the six type II members in the hair follicle and the combined catalog of human type I and II keratins. J. Biol. Chem., 276: 35123-35132.

Marshall, R.C., 1983. Characterization of the proteins of human hair and nail by electrophoresis. J. Invest. Dermatol., 80: 519-524.

Marshall, R.C., D.F.G. Orwin and J.M. Gillespie, 1991. Structure and biochemistry of mammalian hard keratin. Electron Microscopy Rev., 4: 47-83.

McLaren, R.J., G.R. Rogers, K.P. Davies, J.F. Maddox and G.W. Montgomery, 1997. Linkage mapping of wool keratin and keratin-associated protein genes in sheep. Mammalian Genome, 8: 938-940.

Nei, M. and A.K. Roychoudhury, 1974. Sampling variance of heterozygosity and genetic distance. Genetics, 76: 379-390.

Nei, M. and W.H. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA., 76: 5269-5273.

Parsons, Y.M., D.W. Cooper and L.R. Piper, 1994. Evidence of linkage between high-glycine-tyrosine keratin gene loci and wool fibre diameter in a Merino half-sib family. Anim. Genet., 25: 105-108.

Powell, B.C. and G.E. Rogers, 1994. Differentiation in Hard Keratin Tissues: Hair and Related Structures. In: Keralinocyte Handbook, Leigh, F., R.F. Watt and B. Lane (Eds.). Cambridge University Press, Cambridge, pp: 401.

Pruett, N.D., T.V. Tkatchenko, L. Jave-Suarez, D.F. Jacobs and C.S. Potter *et al.*, 2004. Krtap16, charatcterization of a new hair keratin-associated protein (KAP) gene complex on mouse chromosome 16 and evidence for regulation by Hoxc13*. J. Biol. Chem., 279: 51524-51533.

Rogers, G.E. and B.C. Powell, 1993. Organization and expression of hair follicle genes. J. Invest. Dermatol., 10: S50-S55.

SPSS, 1999. SPSS version 10.0 per Windows®. SPSS Inc., Headquarters, Wacker Drive, Chicago, Illinois, USA.

Sambrook, J. and D.W. Russell, 2001. Molecular Cloning: A Laboratory Manual. 3rd Edn., Cold Spring Harbor Laboratory Press, New York, ISBN: 9780879695774, pp: 1-99.