BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, April 2015. Vol. **12**(1), 23-28

Genetic Variation and SNP of Tyrosinase Gene Among Some Camel Breeds Reared in Egypt

Sally S. Alam, Heba A.M. Abd El-Kader, Sekena H. Abd El-Aziem and Othman E. Othman*

Department of Cell Biology, National Research Centre, Dokki, Egypt.

DOI: http://dx.doi.org/10.13005/bbra/1631

(Received: 10 February 2015; accepted: 03 March 2015)

Tyrosinase is a key enzyme in the metabolic pathway leading to coat color pigmentation. Mutations in the tyrosinase (*TYR*) gene are responsible for the albino phenotype in mammals and chicken. Loss of tyrosinase mRNA expression prevents melanin synthesis, thereby causing albinism. The objective of this study was to detect the genetic variations and SNPs of tyrosinase gene among five camel breeds reared in Egypt; Sudany, Somali, Mowaled, Maghrabi and Falahy. Genomic DNA was extracted from blood samples of camels belonging to the five tested breeds and the genotyping of *TYR* was studied using PCR-RFLP technique. The amplified fragment of camel *TYR* exon 1 at 474-bp was digested with the restriction enzyme *Dde*I. The result showed the appearance of three different genotypes; CC, CT and TT in the tested breeds with significant differences in genotype and allele frequencies between these breeds. The camels in Somali, Falahy and Sudany breeds had slightly higher T allele frequency (0.38, 0.36 and 0.33, respectively) than those in Maghrabi and Mowaled camels (0.18 and 0.27, respectively). The genotype TT was detected only in Somali, Falahy and Sudany camels. The overall genotype frequencies for all breeds obtained were 0.06, 0.49 and 0.45 for TT, TC and CC, respectively. The sequence analysis declared the presence of a SNP (C/T) at position 135 in the amplified fragment which is responsible for the destruction of the restriction site C^TCAG and consequently the appearance of two different alleles C and T. The nucleotide sequences of camel *TYR* alleles C and T were submitted in GenBank database and have accession numbers: KP193960 and KP193961, respectively. It is concluded that only one SNP C/T was detected in *TYR* gene among the five tested camel breeds and this nucleotide substitution can be used as a marker for the genetic biodiversity between camels breeds reared in Egypt. Also, due to the possible association between *TYR* gene with coat color pigmentation, we can used its polymorphism for MAS in breeding programs for targeted color camels.

Key words: Camel breeds in Egypt, *TYR,* PCR, RFLP, SNPs.

Oculocutaneous albinism is an autosomal hereditary disorder characterized by a partial or total absence of melanin in the hair, skin and eyes in humans and animals (Damé *et al*., 2012). The synthesis and distribution of melanins from melanosomes in the cytoplasm of melanocytes determine the basic color of these organs in mammals (Zhang *et al*., 2012). Melanocortin receptor 1 (MC1R) locus, mapped to bovine chromosome 18, plays an important role in the regulation of the switch between two types of melanins; eumelanin (black pigment) and phenomelanin (red pigment) in cattle (Klungland and Vage, 2003 and Seo *et al*., 2007).

Tyrosinase is identified as the key enzyme in the melanogenesis pathway where its high level leads to the production of eumelanin while its low level directs to phenomelanin (Gutierrez-Gil *et al*., 2007). Tyrosinase (*TYR*) gene is expressed in melanocytes and controls the major steps in pigment production (Renugadevi *et al*., 2010). This gene is of interest in farm animals because of its

* To whom all correspondence should be addressed.
Fax: 202 33370931;
E-mail: othmanmah@yahoo.com

role in coat color production where it has been shown to cause a range of dilution phenotypes including complete albinism in cattle, humans, mice and chickens. There were about 150 different *TYR* mutations characterized in human (Oetting and King, 1999), cattle (Schmutz *et al*., 2004), rabbit (Aigner *et al*., 2000) and mice (Beermann *et al*., 2004).

The Camel is known to be a multipurpose animal (Hjortaf Ornäs and Hussain, 1993) able to produce milk, meat, wool, hides and skins, to be used for riding, agricultural activities, packing and carting, racing and many cultural events. There is probably not another domestic animal as versatile for the human population, especially those living in arid lands (Faye and Bonnet, 2012).

In Egypt, the camel population was about 120 thousand head, (SADS, 2009). The main camel breeds reared in Egypt are Maghrabi, Falahy, Sudany, Somali and Mowaled (Mahrous *et al*., 2005). Egyptian camels are important animals because they are dual purpose animals (meat and milk). In the Nile Valley and Delta, they are mainly raised for meat production whereas in the desert, they are raised equally for meat and milk production, some labors and transport.

With the little interesting regarding to the genetically characterizations of camel breeds in Egypt, the objectives of this work was the detection of genetic variations of *TYR* gene in five camel breeds reared in Egypt using RFLP and also the identification of SNPs which are responsible for these genetic variations.

MATERIAL AND METHODS

Blood samples and genomic DNA extraction

Blood samples were collected form jugular vein of 15 individual camels from each tested breed; Fallahi, Maghrabi, Mowalled, Sudany and Somali. Genomic DNA was extracted from the whole blood according to the method described by Miller *et al*. (1988) with minor modifications. Briefly, Blood samples were mixed with cold 2x sucrose-triton and centrifuged at 5000 rpm for 15 min at 4°C. The nuclear pellet was suspended in lysis buffer, sodium dodecyl sulfate and proteinase K and incubated overnight in a shaking water bath at 37°C. Nucleic acids were extracted with saturated NaCl solution. The DNA was picked up and washed in 70% ethanol. The DNA was dissolved in 1x TE buffer. DNA concentration was determined, using Nano Drop1000 Thermo Scientific spectrophotometer and then diluted to the working concentration of 50ng/µl which is suitable for polymerase chain reaction.

Polymerase chain reaction

The primers used in this study were designed on the basis of DNA sequence of the camel tyrosinase gene (shah *et al*., 2012),

F: 5' > AGC CTG TGC CTC CTC CAA GAA < 3'

R: 5' > TGC ATC CAT ACA AAG AAG TCA TAA < 3'

A PCR cocktail consisted of 1.0 mM upper and lower primers specific for tyrosinase gene, 0.2 mM dNTPs, 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 % gelatin (w/v), 0.1 % Triton X-100 and 1.25 units of Taq polymerase. The cocktail was aliquot into PCR tubes with 100 ng of camel DNA. The reaction was run at 94°C for 5min, 35 cycles of 94°C for 1min, 55°C for 30s, 72°C for 45s and a final extension at 72°C for 5min. The PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide to test the amplification success.

RFLP analysis

To detect variants of *TYR* gene in different camel breeds, the PCR products were digested using restriction enzyme; *Dde*I (Fermentas). Ten µl of PCR product was digested with 1µl of FastDigest restriction enzymes for 5min at 37°C. The restriction fragments were subjected to electrophoresis in 2% agarose/ethidium bromide gel (GIBCO, BRL, England) in 1x TBE buffer (0.09 M Tris-boric acid and 0.002 M EDTA). Gels were visualized under UV light and documented in FX Molecular Imager apparatus (BIO-RAD).

DNA Sequencing and sequence analysis

The PCR products -representing each detected genotype of *TYR* gene in different camel breeds- were purified and sequenced by Macrogen Incorporation (Seoul, Korea). Sequence analysis and alignment were carried out using ClustalW2 to detect each single nucleotide substitution between two different detected alleles. Results of endouclease restriction were carried out using Fast PCR. The nucleotide sequences of different alleles for camel *TYR* gene were submitted to GenBank (NCBI, BankIt).

RESULTS AND DISCUSSION

Currently, the primary thrust of research in animal genetics is the identification of genes, which affect the expression of quantitative traits markedly. Developments in DNA technologies have made it possible to uncover a large number of genetic polymorphisms at the DNA sequence level, and to use them as markers for the evaluation of the genetic basis for the observed phenotypic variability (Teneva, 2009).

The tyrosinase gene has long been implicated in the coat color determination. This gene is of interest in farm animals because of its role in coat color production. It has been shown to cause a range of dilution phenotypes including complete albinism in cattle, humans, mice and chickens (Schmidtz *et al*., 2001). Tyrosinase gene has been reported to be consisting of 5 exons and 4 introns in mammals (Giebel *et al*., 1991). We aimed in this study to genetically characterization of *TYR* gene in some camel breeds reared in Egypt.

The primer of tyrosinase gene which used in this study flanked 474-bp fragment from exon 1 of this gene (Shah *et al*., 2012). The amplified fragments obtained from tested camels (Fig. 1) were digested with *Dde*I endonuclease to detect the *TYR* variations between camels belonging to the five tested breeds. The digestion results showed the appearance of 3 different genotypes (Fig. 2) according to the presences of one or two

Table 1. Genotype and allele frequencies of the tyrosinase gene in different camel breeds

Breed	Genotype frequencies			Allele frequencies	
	CC	CT	TT	C	T
Falahy	0.36	0.57	0.07	0.645	0.355
Maghrabi	0.64	0.36	0.00	0.82	0.18
Mowaled	0.45	0.55	0.00	0.725	0.275
Somali	0.39	0.46	0.15	0.62	0.38
Sudany	0.42	0.50	0.08	0.67	0.33
Total	0.45	0.49	0.06	0.70	0.30

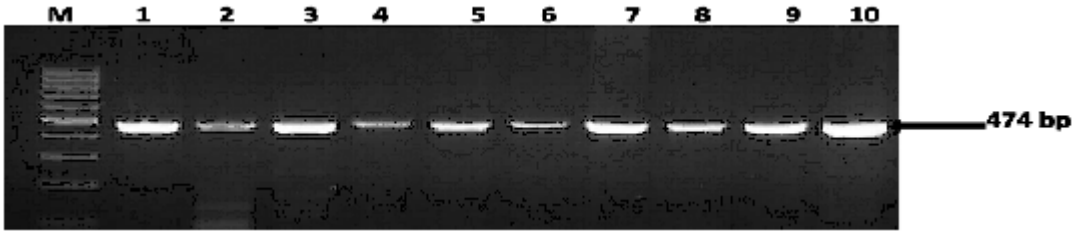

Fig. 1. Electrophoretic pattern of PCR-amplified fragment from *TYR* gene in camels. M: 100-bp ladder marker. Lanes 1-10: 474-bp PCR product amplified from camel DNA

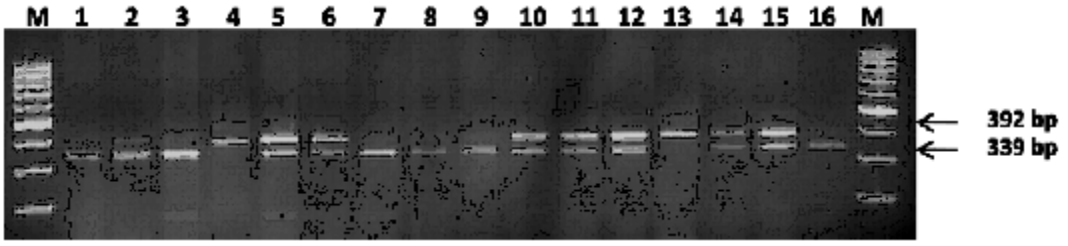

Fig. 2. Different genotypes obtained after digestion of PCR products of camel *TYR* gene with *Dde*I. Lane M: 100-bp ladder marker. Lanes 1, 3, 7-9 and 16: Homozygous genotype CC. Lanes 2, 5-6, 10-12, 14-15: Heterozygous genotype CT. Lanes 4 and 13: Homozygous genotype TT. The small-sized fragments 82- and 53-bp do not showed in the figure.

restriction sites of *Dde*I (C^TNAG) (Fig. 3). These genotypes were homozygous TT with two digested fragments at 392- and 82-bp; homozygous CC with three digested fragments at 339-, 82- and 53-bp and heterozygous genotype CT with 4 digested fragments (392-, 339-, 82- and 53-bp).

Table 1 showed the allele and genotype frequencies of tested camels, from which we noticed that all breeds possessed the three detected genotypes in different frequencies with the exception of the Mowaled and Maghrabi breeds which lack TT genotypes. The highest frequency of CC genotype was in Maghrabi breed (0.64), TT in Somali breed (0.15) and CT genotype in Falahy breed (0.57). The overall frequencies of C and T alleles in tested camel breeds were 0.7 and 0.3, respectively.

In agreement with our results, Shah *et al*. (2008 and 2012) reported a significant differences in genotype and allele frequencies among 6 different Pakistani camel breeds (Marecha, Dhatti, Larri, Kohi, Campbelpuri and Sakrai) where the genotype frequencies ranged from 0.19-0.40, 0.33-0.56 and 0.04-046 for TT, CT and TT genotypes, respectively with the dominant of allele C over allele T as detected in our work.

Ishag *et al*. (2013) detected the allelic variants of *TYR* gene exon 1 in six Sudanese camel breeds; Kenani, Lahwee, Rashaidi, Anafi, Bishari and Kabbashi and also investigated the possibility of associations between SNPs of TYR with coat color and body measurements in these breeds. In spite of the significantly affect of *TYR* genotypes on shoulder height, there is no significantly influence on other phenotypic measurements or on Sudanese camel coat color. The genotype frequencies for all tested breeds were 0.02. 0.25 and 0.72 for TT, TC and CC genotypes, respectively. These reported frequencies match with those of our finding and previous studies where the highest genotype frequency was for CC followed CT then TT and the dominant of allele C over allele T.

Our result suggested a possible association between *TYR* allele T and the darkness camel coat color where the highest frequencies of this allele were present in Somali (0.38), Falahy (0.355) and Sudany (0.33) and the camels of these breeds possess darkness color ranged from brown to reddish brown. On contrary, the animals of the other two tested breeds which have lowest frequencies of T allele; Mowaled (0.275) and Maghrabi (0.18) possess creamy or pale color. This suggestion was supported by finding of Shah *et al*. (2008 and 2012) where they reported the presence of T allele with high frequency in Pakistani Sakrai and Campbelpuri breeds which their camel color ranges from reddish brown to dark brown and blackish.

Oculocutaneous albinism has previously been reported in an Anatolian buffalo calf in Turkey (Coban and Yildiz, 2005) and in a herd of Murrah buffalo in Brazil (Marcolongo-Pereira *et al*., 2010). Damé *et al*. (2012) reported the clinical and genetic features of albinism in a herd of water buffalo of the Murrah breed. The results of segregation analysis suggest that this disease is acquired through recessive inheritance. In the OCA buffalo, a single-base substitution was detected at nucleotide 1,431 (G to A), which leads to the conversion of tryptophan into a stop codon at residue 477. This premature stop codon produces an inactive protein, which is responsible for the OCA buffalo phenotype.

In another study, Zhang *et al*. (2012) screened and characterized the genetic

Fig. 3. Endonuclease restriction of camel *TYR* amplified fragments using Fast PCR. C^TCAG restriction sites with red

polymorphism in genomic DNA of *TYR* exon 2 and the 5´-UTR in Tianzhu white yak to identify possible causative and functional mutations in *TYR* gene responsible for the white coat color in yak. They reported the identical sequence for exon 2 in white and black yak populations whereas there are 14 genotypes and seven alleles in 215-bp amplified fragment of the 5´-UTR without suggested association between them and yak coat color.

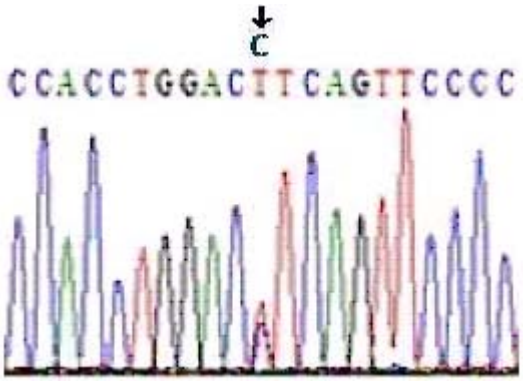

Fig. 4. C/T substitution in camel *TYR* exon 1

The nucleotide sequences of different detected genotypes in our result declared the presence of one SNP substitution (C/T) at position 135 in the amplified fragments of camel *TYR* exon 1 (Fig. 4) which is responsible for the destruction of the restriction site C^TCAG and consequently the appearance of two different alleles C and T (Fig. 5). The nucleotide sequences of camel *TYR* alleles C and T were submitted in GenBank database and have accession numbers: KP193960 and KP193961, respectively.

It is concluded that only one SNP C/T was detected in *TYR* gene exon 1 among the five tested camel breeds and this nucleotide substitution can be used as a marker for the genetic biodiversity between camel breeds reared in Egypt. Also, due to the possible association between *TYR* polymorphism with coat color pigmentation, we

```
Allele C   AGCCTGTGCCTCCTCCAAGAACTTGATGGAGAAGGAATGCTGCCCGCCGTGGGAGGGTGA  60
Allele T   AGCCTGTGCCTCCTCCAAGAACTTGATGGAGAAGGAATGCTGCCCGCCGTGGGAGGGTGA  60
           ************************************************************

Allele C   CGGGAGTCCCTGTGGCCAGCTCTCAGGCAGGGGTTCCTGTCAGGACATCAATCTGTCCAA  120
Allele T   CGGGAGTCCCTGTGGCCAGCTCTCAGGCAGGGGTTCCTGTCAGGACATCAATCTGTCCAA  120
           ************************************************************

Allele C   GGCACCACCTGGACCTCAGTTCCCCTTCACAGGGGTGGATGACCGGGAATCTTGGCCCTC  180
Allele T   GGCACCACCTGGACTTCAGTTCCCCTTCACAGGGGTGGATGACCGGGAATCTTGGCCCTC  180
           ************** ************************************************

Allele C   TGTCTTTTATAACAGGACCTGCCAGTGCTTTGACAACTTCATGGGATTCAACTGTGGAAA  240
Allele T   TGTCTTTTATAACAGGACCTGCCAGTGCTTTGACAACTTCATGGGATTCAACTGTGGAAA  240
           ************************************************************

Allele C   TTGCAAGTTTGGCTTCCGGGGACCCAACTGCAGAGAGAGGCGACTTTTGGTGAGAAGAAA  300
Allele T   TTGCAAGTTTGGCTTCCGGGGACCCAACTGCAGAGAGAGGCGACTTTTGGTGAGAAGAAA  300
           ************************************************************

Allele C   CATCTTTGATTTGAGTGTCCCAGAGAAGAACAAATTTCTTGCCTACCTCACTTTAGCCAA  360
Allele T   CATCTTTGATTTGAGTGTCCCAGAGAAGAACAAATTTCTTGCCTACCTCACTTTAGCCAA  360
           ************************************************************

Allele C   ACATACCACCAGCCCAGACTACGTCATCCCCACGGGCACCTATGGCCAAATGAATAATGG  420
Allele T   ACATACCACCAGCCCAGACTACGTCATCCCCACGGGCACCTATGGCCAAATGAATAATGG  420
           ************************************************************

Allele C   ATCAACACCCATGTTCAATGACATCAACGTTTATGACCTCTTTGTATGGATGCA  474
Allele T   ATCAACACCCATGTTCAATGACATCAACGTTTATGACCTCTTTGTATGGATGCA  474
           ********************************************************
```

Fig. 5. Nucleotide sequences and alignment between *TYR* alleles C and T using ClustalW2, C/T substitution at position 135

can use its polymorphism in MAS for targeted color camel breeding programs.

REFERENCES

1. Aigner, B., Besenfelder, U., Muller, M. , Brem, G.: Tyrosinase gene variants in different rabbit strains. *Mamm. Genome,* 2000;**11**: 700-702.
2. Beermann, F., Orlow, S.J., Lamoreux, M.L.: The Tyr (albino) locus of the laboratory mouse. *Mamm. Genome,* 2004; **15**: 749-758.
3. Coban, O., Yildiz, A.: Albinism in an anatolian buffalo calf. *J. Appl. Anim. Res.,* 2005; **27**: 61-62.
4. Damé, M.C.F., Xavier, G.M., Oliveira-Filho, J.P., Secorun Borges, A.S., Oliveira, H.N., Riet-Correa, F., Schild, A.L.: A nonsense mutation in the tyrosinase gene causes albinism in water buffalo. *BMC Genetics,* 2012 ;**13**: 62-68.
5. Faye, B., Bonnet, P.: Camel sciences and economy in the world: Current situation and perspectives. 3rd Conference of the International Society of Camelid Research and Development. 29 Jan- 1 Feb ,2012, Muscat, Sultanate of Oman.
6. Giebel, L.B., Strunk, K.M., Spritz, R.A. : Organization and nucleotide sequences of the human tyrosinase gene and a truncated tyrosinase-related segment. *Genomics,* 1991; **9**: 435-445.
7. Gutierrez-Gil, B.,Wiener, P., Williams, J.L. : Genetic effects on coat color in cattle: Dilution of eumelanin and phaeomelanin pigments in an F2-backcross Charolais ´ *Holstein population. BMC Genet.,* 2007; **8**: 56.
8. Hjört af Ornäs, A., Hussein, M.A. : Camel herd dynamics in southern Somalia: long term development and milk production implications. . In: A. Hjort af Ornäs (Ed.). The multipurpose camel: interdisciplinary studies on pastoral production in Somalia., EPOS, Uppsala University, Sweden, 1993; 31-42.
9. Ishag, I.A., Reissmann, M., Eltaher, H.A., Ahmed, M.K.A. : Polymorphisms of Tyrosinase gene (Exon 1) and its impact on coat color and phenotypic measurements of Sudanese Camel Breeds. *Sci. J. An. Sci.*, 2013; **2**(5): 109-115.
10. Klungland, H. , Vage, D.I. : Pigmentary switches in domestic animal species. *Ann. N.Y. Acad. Sci.,* 2003; **994**: 331-338.
11. Mahrous, K.F., Abd-El Mordy, M., Abd El-Aziem, S.H., Hemdan, D.M. : Genetic variations in one-humped camels present in Egypt. *J. Genet. Eng. Biotech., (NRC),* 2005; **3**(1): 15-29.
12. Marcolongo-Pereira, C., Schild, A.L., Soares, M.P., Vargas, S.F., Riet-Correa, F. : Congenital defects in ruminants in southern Brazil. Pesq. *Vet. Bras.,* 2010; **30**: 816-826.
13. Miller, S.M., Dykes, D.D., Polesky, H.F. : A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.,* 1988; **16**(3): 1215.
14. Oetting, W.S. , King, RA. : Molecular basis of albinism: mutations and polymorphisms of pigmentation genes associated with albinism. *Hum. Mut.*,1999; **13**(2): 99-115.
15. Renugadevi, K., Sil, A.K., Perumalsamy, V., Sundaresan, P.: Spectrum of candidate gene mutations associated with Indian familial oculocutaneous and ocular albinism. *Mol. Vision,* 2010; **15**: 1514-1524.
16. SADS (Sustainable Agricultural Development Strategy Towards 2030) : Agricultural Research & Development Council. Arab Republic of Egypt, Ministry of Agriculture and Land Reclamation. Oct.,2009.
17. Schmidtz, B.H., Buchanan, F.C., Plante, Y., Schmutz, S.M. : Linkage mapping of tyrosinase gene to bovine chromosome 26. *Anim. Genet.,* 2001; **32**: 119-120.
18. Schmutz, S M., Berryere, T.G., Ciobanu, D.C., Mileham, A.J, Schmidtz, B.H., Fredholm, M. : A form of albinism in cattle is caused by a tyrosinase frame shift mutation. *Mamm. Genome,* 2004; **15**: 62-67.
19. Seo, K., Mohanty, T.R., Choi, T., Hwang, I. : Biology of epidermal and hair pigmentation in cattle. A mini-review. *Vet. Dermatol.,* 2007; **18**; 392-400.
20. Shah, M.G., Reissmann, M., Qureshi, A.S., Schwartz, D.H.J. : Evaluation of six camel breeds for heterozygocity through restriction fragment length polymorphism. *Pak. Vet. J.*, 2008; **28**(1): 13-16.
21. Shah, M.G., Reissmann, M., Qureshi, A.S., Schwartz, H.J., Khan, M.S., Ali, T., Zaman, A., Safdar, K., Khalid Muhammad K., Ullah, S. : Determination of genotype differences through restriction endonuclease in Camels (*Camelusdromedarius*). *Afr. J. Biotechnol.,* 2012; **11**(53): 11543-11547.
22. Teneva, A. : Molecular markers in animal genome analysis. Biotechnology in Animal Husbandry, 9-th International Symposium Modern Trends in Livestock Production, *Belgrade*, 2009; 1267-1285.
23. Zhang, H., An, T.W., He, J.W., Luo, Y.Z., Han, J.L.: Conserved exon 2 but a highly polymorphic 5'-UTR of tyrosinase gene in Tianshu White Yak (Bos grunniens). *Asian J. Anim. Vet. Adv.,* 2012; **7**(11): 1090-1099.