

Expression of the Tyrosinase Gene in Different Dromedary Camels of Saudi Arabia

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ABSTRACT

Tyrosinase (*TYR*) gene has a role in regulating the pigmentation process and acts as a precursor for eumelanin or pheomelanin. There have been many studies that reported variations in this gene associate with the coat color of the animal. This is the first study of *TYR* gene expression analysis from various dromedary or Arabian camel (*Camelus dromedarius*) phenotypes from Saudi Arabia. The four camel groups included were white, diluted (light brown, creamy and fawn), black and dark brown representing eumelanin and pheomelanin coat colors. Generally, the *TYR* gene expresses less in non-pigmented than the pigmented species. The results of our study showed that the mRNA from skin biopsies has low expression in white dromedary camels while higher expression in dark brown phenotypes followed by black and diluted ones through real time quantitative PCR. MicroRNAs (miRNAs) miR-129-5p and miR-145 were evaluated for their depigmentary role and observed reciprocal expression against the *TYR* gene expression. Hence the miR-129 and miR-145 expressed higher in non-pigmented camels compared to the pigmented ones and correlated with *TYR* expression. Our data provides useful information to understand the camel *TYR* role in coat color variation which is supportive in genotyping, genetic selection, breeding and classification.

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Authors' Contribution

AS designed the work, AS and HI carried out the experiments. FA supervised the work and analyzed the data. AS, FA and HI finalized the manuscript.

Key words

Tyrosinase, Coat color, Dromedary camel, Micro RNA, miR-145.

INTRODUCTION

Dromedary camel is an important livestock animal adapted to harsh desert environments (Eshra and Badawy, 2014). It is raised for milk, meat and wool production and its demand is increasing globally therefore integrating into the international economy (Faye and Bonnet, 2012; Faye, 2018). It is useful in agriculture, transport, racing, beauty contest and tourism. In Saudi Arabia, there are different phenotypic camels in various regions and they are mainly used in milk production, meat production and racing: black (majahim), white (wodh), brownish yellow (safrah), yellowish to red (hadahana), red to white (awadi), white (awarik), brown (hamrah), red (saheli), grey (shaele), grey (shageh), dark brown (sofor) and blue grey (zargeh) (Abdallah and Faye, 2012; Mahmoud et al., 2020).

It is important to characterize the coat color genes completely to understand their role due to the commercial value of specific phenotypes and breeding purposes.

Tyrosinase is an enzyme that catalyzes the biosynthesis of melanin through various metabolic pathways producing quinone, which is the precursor for melanin pigmentation. It is encoded by the *TYR* gene and present in specialized cells such as skin, hair and eyes to provide pigmentation. It is present on the 10th chromosome of the dromedary camel and spanning over 5 exons. It is generally known as albino locus due to variations in this gene cause Albinism in various species (Anello et al., 2019). There have been reported more than 100 mutations in the *TYR* gene with albinism due to the absence of melanin resulting in white color of hair and skin as well as the pale skin. It has been reported in other species including mice, cats, cattle, rabbits, buffalos, donkeys and others (Yokoyama et al., 1990; Beermann et al., 2004; Aigner et al., 2000; Imes et al., 2006; Damé et al., 2012; Schmutz et al., 2004; Utzeri et al., 2016). In this gene, there were some repeated sequences (Microsatellites) in the noncoding DNA, which may have a role in the gene transcription and phenotype pigmentation (Li et al., 2004; Sjakste et al., 2013).

The SNP c.428 A > G was higher in white coat color than other phenotypes (Anello et al., 2019). A point mutation in the *TYR* gene of chinchilla mice observed with greyish color (Lamoreux et al., 2001) and another point

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mutation resulted in platinum type phenotype (Orlow *et al.*, 1993). Hypopigmentation was also observed in the body and eyes of mice (Challa *et al.*, 2016). Some other mutations in this gene reported diluted phenotypes (Himalayan and chinchilla mice).

Some of the studies including Sudanese camel breeds, Egyptian, Pakistani and Saudi Arabian reported with the presence of different genotypes and snp's in the exon 1 of this gene (Mahmoud *et al.*, 2020; Ishag *et al.*, 2013; Nowier *et al.*, 2020; Shah *et al.*, 2009, 2012). One of the studies reported Arabian camels were classified based on their different phenotypic coat colors such as white, black, brown and dark brown (Almathen *et al.*, 2018). A recent study on llamas has been reported with *TYR* gene polymorphism with regards to their coat color phenotypes (Anello *et al.*, 2019). Different gene studies were carried out in camels to study the pigmentation variation in coat colors such as MC1R, ASIP (Daverio *et al.*, 2016; Almathen *et al.*, 2018), KIT (Holl *et al.*, 2017). The variations in the coat color might be influenced by epigenetic factors such as methylation, miRNA and chromatin modifications. In this study, the involvement of miRNAs against *TYR* gene was evaluated.

MiRNAs are conserved, small and noncoding RNA's which regulate the gene expression by inhibiting the target mRNA at the post-transcriptional level. They bind to the target mRNA and inhibit the protein synthesis or overexpress the protein. Different miRNAs have been reported as regulating the melanogenesis such as miR-434-5p and miR-25 were used to target *TYR* and *MITF*, respectively (Wu *et al.*, 2008; Zhu *et al.*, 2010). MiR-145 has been reported with depigmentary activity by degrading the *TYR* mRNA expression in human and mouse melanocytes (Dynoodt *et al.*, 2013). Therefore, its

expression increased more folds in depigmented animals and decreased in pigmented animals. Similarly, miR-129-5p was expressed low in Inner Mongolia cashmere goats (white) and higher in Dazu black goats (pigmented) by regulating the *TYR* gene (Li *et al.*, 2020). Naturally, miR-129-5p along with 53 other miRNAs that influence the pigmentation by regulating the *TYR* gene (Wu *et al.*, 2008).

In this study, we analyzed *TYR* gene expression and its association with the coat colors of the different dromedary camels (white, black, diluted and dark brown) and investigated the miRNAs (129-5p and 145) to correlate with *TYR* mRNA expression.

MATERIALS AND METHODS

Sample collection

Animal ethics approval was obtained from the deanship of scientific research, King Faisal University. Dromedary camel samples (n=16) white (wodh) (4), black (majahim) (3), dark brown (sufor) (4) and diluted (shual) (5) including male and female were selected based on their phenotypic variation from the Camel Research Center at King Faisal University is presented in [Supplementary Figure S1](#). Animals prepared aseptically with iodine, shaved the selected area and marked. Lidocaine (2%) was given as the local anesthesia subcutaneously then waited for 10 min. Robbins Instruments Disposable Biopsy Punches (3 Mm) were used to collect the skin samples by rotating gently without applying pressure and cutting the sample with surgical scissors. Iodine and antiseptic powder was applied to the wound and it was monitored further to check for any infections. The collected samples were placed in sterile tubes with icebox and transferred to the lab and stored at -20°C until further processing.

Table I.- Primers used in PCR.

S. No	Name	Primers (5'→3')	Annealing temp. (°C)	Product length (bp)
1	<i>TYR</i>	F: TCAGCTTTAGAAACACCCTGGA R: CTTCAAGCCACTGTTCAAAAA	58.5	196
2	<i>GAPDH</i>	F: CCATGTTTGTGATGGGCGTG R: CATCTCCAGAGGGGCCAT	57.5	200
3	MiR-129	F: CTCAACTGGTGTCTGAGTCCGGCAATTCAGTTGAGAGCAAGCC R: AACTCCAGCTGGGCTTTTTCGG	-	-
4	MiR-145	F: CTCAACTGGTGTCTGAGTCCGGCAATTCAGTTGAGAGGGATTC R: AACTCCAGCTGGGGTCCAGTTTT	-	-
5	U6 (internal control)	F: GCTTCGGCAGCACATATACTAAAAT R: CGCTTCACGAATTTGCGTGCAT	-	-
6	Universal primer	AACTGGTGTCTGAG	-	-

GADPH, glyceraldehyde-3-phosphate dehydrogenase; -, not applicable.

RNA isolation, cDNA and real-time PCR

To study the *TYR* gene expression, skin biopsy samples from four coat color groups were obtained and followed the RNA Trizol® procedure was used to extract the total RNA from the skin biopsies and measured the RNA quality and quantity on nanodrop. cDNA was prepared with reverse transcriptase (Applied Biosystems, Vilnius Lithuania). Quantitative realtime PCR was accomplished by using a set of designed primers (Table I), the exon-exon region of the GAPDH housekeeping gene used as a control. The 12µl of the mix was prepared with PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, Warrington, UK) (6µl), ~500 nM of primers, and cDNA as per the concentration and ran on PCR (Applied Biosystems ViiA 7 Real-Time PCR System). The program set for PCR was as follows; 50°C – 2 min, 95°C – 10 min, 40 cycles of 95°C – 15 sec, 60°C – 1 min and final gradient 95 to 60°C. The mRNA quantification of *TRY* and the endogenous control GAPDH with the fold change in the target mRNAs were calculated using the comparative $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001). We selected miR-129-5p and miR-145 from the bioinformatic program TargetScan (<http://www.targetscan.org>) and synthesized from the Macrogen Inc., Korea (Table I).

RESULTS

TYR gene of the dromedary camel

We assessed the *TYR* gene of Saudi Arabian dromedary camels representing different coat colors. It has the predicted coding sequence of 1724 bp and encodes a *TYR* protein with 574 amino acids. This protein is 99.67% identical with *Camelus ferus* (XM_006192400.2) and 99.55% with *Camelus bactrianus* (XM_010973375.1).

TYR expression in different dromedary camel phenotypes

The effect of *TYR* mRNA on camel coat color was measured using quantitative real time-PCR. The 300 ng of extracted RNA was used for cDNA preparation. This complete cDNA was quantified and used for the investigation of *TYR* expression. The results showed *TYR* gene expression was significantly higher in dark brown followed by black, diluted and lowest recorded in white dromedary camels representing higher expression in pigmented ones (Fig. 1). Diluted color strains have 8.5 fold higher expression against white camel species and it was significantly overexpressed ($p < 0.003$). Moreover, the *TYR* mRNA expression was increased 7 fold in black camels compared to diluted strain camel ($p < 0.001$). A similar overexpression was observed in dark brown camels compared to black camel species. From the above findings, Dark brown has >600 fold higher *TYR* mRNA

expression in when compared to white dromedary camel species (Fig. 1). Hence, it is clear that *TYR* mRNA was down regulated in non-pigmented individuals than the pigmented ones. It was further confirmed by post-transcriptional influencing factors.

Identification and selection of miRNAs–miR-129-5p and miR-145

The identification of *TYR* mRNA targeted miRNAs were by using a Targetscan tool. The preliminary findings showed a lack of miRNA evidence in dromedary camel species. So, the studies screened a ruminant based organism known as *Bos taurus* species. *TYR* mRNA sequences were used for the identification of miRNA and found 23 hits of miRNAs (http://www.targetscan.org/cgi-bin/targetscan/vert_72/view_gene.cgi?rs=ENST00000263321.5&taxid=9913&showcnc=0&shownc=0&shownc_nc=&showncf1=&showncf2=&subset=1). The evaluation of miRNA conservation score showed that, miR-129-5p and miR-145 has >8 mer conservation and high context++ score compared to other miRNAs. Whereas, these two miRNAs were further analyzed for miRNA expression in various dromedary camel species. These findings are the first report on camel species.

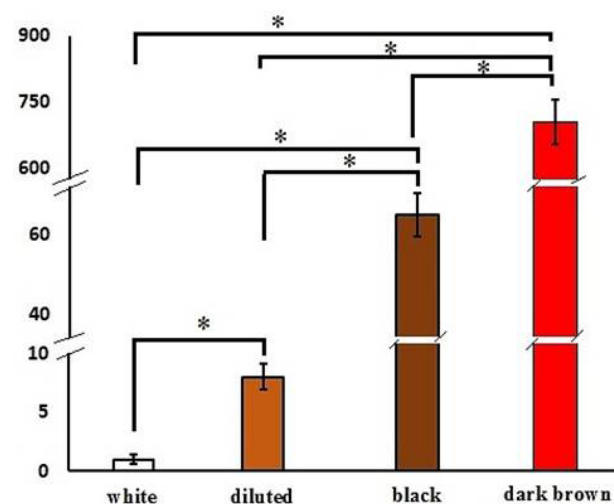


Fig. 1. *TYR* gene expression in skin biopsies of different phenotypic dromedary camels. Bar graphs showing increasing expression from white to dark brown phenotypes. * defines the statistically significant ($p < 0.05$).

MiR-129-5p and MiR-145 expression and their targeted mRNA

The identified miRNAs were evaluated of expression analysis. The two miRNAs (*MiR-129-5p* and *MiR-145*) were similar in expression against the mRNA of *TYR*.

They showed high expression in non-pigmented (where *TYR* is less) and low expression in pigmented camels (where *TYR* is high) as shown in Figure 2. The decreased expression order follows as diluted > white > black > dark brown for miR-129-5p (Fig. 2A, B) and white > diluted >

black > dark brown for miR-145 (Fig. 2C, D). These two miRNAs bind to the mRNA of the *TYR* gene at 3' UTR regions and downregulate the *TYR* expression (Fig. 1). The binding of miRNAs to the *TYR* mRNA is shown in Figure 3.

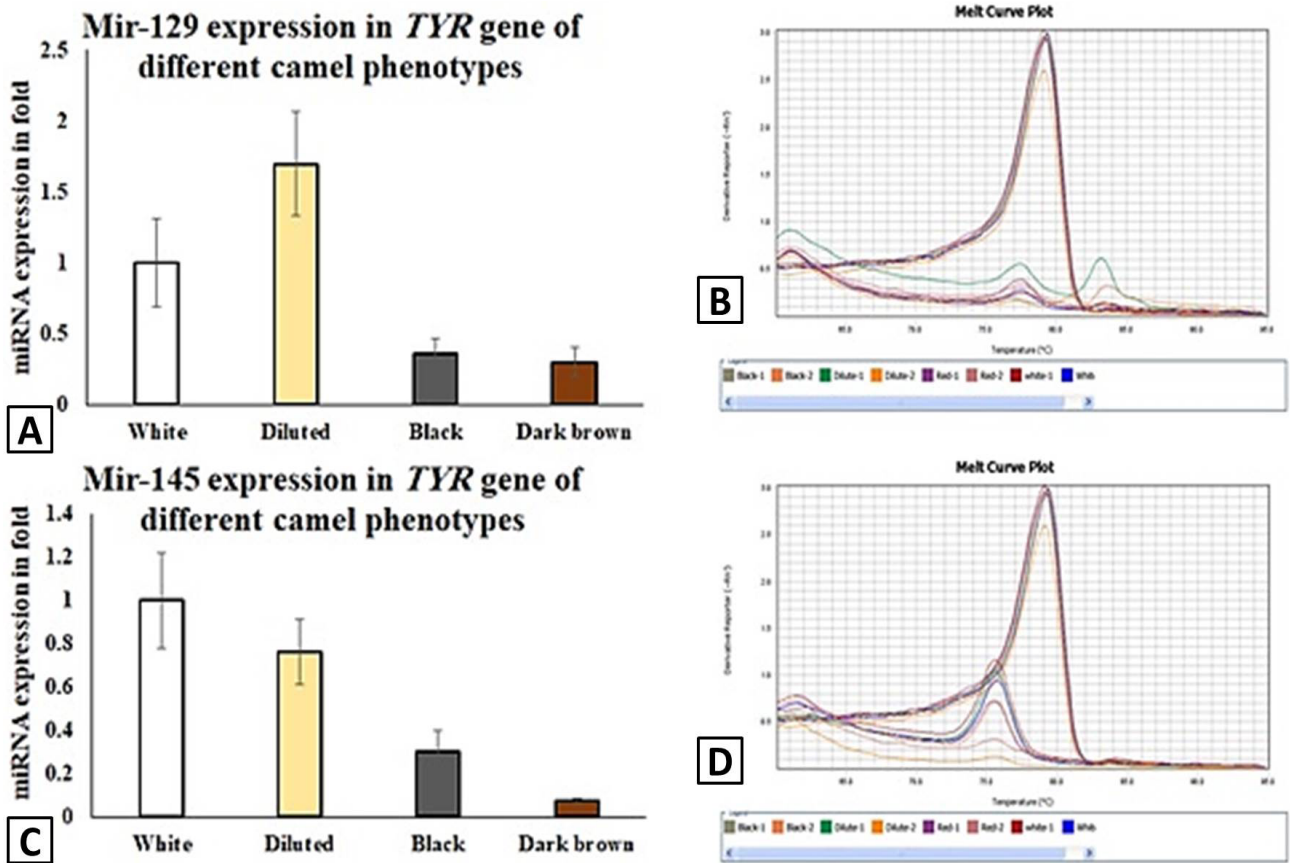


Fig. 2. A, MiR-129-5p expression in different phenotypic dromedary camels showing highest in diluted and lowest in dark brown camels. B, MiR-129 melt-curve showing uniform expression at 790°C. C, MiR-145 expression in different phenotypic dromedary camels showing highest expression in white and significantly low in dark brown camels. D, MiR-145 melt-curve showing uniform expression at 790 C.



Fig. 3. MiRNAs binding to the *TYR*. A, XM_010979466.2-*TYR* protein and miRNA; B, sequence matching; C, Position of binding; *, identical, €, groups with weak similarity.

DISCUSSION

This is the first study as per our knowledge to evaluate the *TYR* gene expression level in different dromedary phenotypic camels. The *TYR* gene involve in melanogenesis pathway switching to various coat colors and this is a conserved gene similar to that of humans and other species.

Some of the mutations in the *TYR* gene have been reported to alter the gene expression and phenotype color such as albino animals in various species (Anistoroaei *et al.*, 2008; Blaszczyk *et al.*, 2005; Schmutz *et al.*, 2004; Imes *et al.*, 2006). Missense mutations in 7 alleles of mice developed hypopigmentation (Challa *et al.*, 2016). A hypopigmented minks were observed with the Himalayan allele due to the presence of snp (Benkel *et al.*, 2009). We assessed the *TYR* gene of different phenotypic dromedary camels (white, black, diluted, and dark brown) for expression patterns.

We did the expression analysis of the *TYR* gene and observed with low expression in white phenotypes and increasing expression from diluted > black > dark brown coat colors. This might be due to the lesser expression of the Microphthalmia-associated transcription factor type M (*MITF-M*) gene which regulates the *TYR* gene (Anello *et al.*, 2019). A very similar result was observed in llamas study by Anello *et al.* (2019) and Paterson *et al.* (2015) in mice phenotypes (Paterson *et al.*, 2015). One of the studies on alpacas reported that the *TYR* gene along with the 7 other color related genes was expressed low in white, intermediate in the bay and high in black phenotypes (Munyard, 2013). Some of the similar studies of *TYR* gene expression in Korean cattle and Jining goats revealed that the pigmented ones expressed higher than the lighter or diluted phenotypes (Kim *et al.*, 2014; Chen *et al.*, 2012). In our study, the dark brown phenotypes showed higher expression than the black which could be due to less sample size and difference in expression. However, there could be similar mutations under various genetic environments that produce different genotypes as it was suggested in different strains of rabbits (Aigner *et al.*, 2000). Moreover, other mutations may influence the actual coat color of the individual. Due to the less exploration and unavailability of complete sequencing data of this gene such as total exon sequencing it was not possible to correlate completely. Further study with more phenotypic samples and complete sequencing could be carried out to correlate with the coat color. *TYR* is one of the key gene among other genes involved in melanin synthesis and rate-limiting enzyme. The MITF is the vital transcription factor for the *TYR* gene which induces the tyrosinase-related protein 1 (*TYRPI*) and lead to the process of melanogenesis. Apart from the

transcription factors and melanin synthesizing genes there are some miRNAs which have a significant regulatory role in melanin biosynthesis. A few of them are miR-129-5p, miR-145, miR434-5p, miR-25 which target the mRNA of *TYR* and MITF and downregulate or upregulate the melanin synthesis (Dynoodt *et al.*, 2013; Li *et al.*, 2020; Wu *et al.*, 2008).

We selected miR-129-5p and miR-145 to evaluate the *TYR* expression. They were inversely proportional to the mRNA of the *TYR* gene expression. They bind to the mRNA of *TYR* gene and upregulate or downregulate its expression. Therefore, these two miRNAs were highly expressed in white and diluted phenotypes and low in black and dark brown ones (Fig. 2). This was consistent with the previous studies (Dynoodt *et al.*, 2013; Li *et al.*, 2020; Wu *et al.*, 2008). Hence, it was correlated that the *TYR* expression with the miRNAs expression.

CONCLUSION

In this study, we analyzed the *TYR* gene of different phenotypic dromedary camels for its expression and association with the various coat colors. It was less expressed in white than the pigmented ones which were further evaluated with miRNAs expression. This study also revealed that the understanding of *TYR* gene expression and its influence on melanogenesis via non-coding miRNA regulatory markers. Our data are useful in understanding the phenotypic classification of the coat color.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20210204120257>

Statement of conflict of interest

The authors have declared no conflict of interests.

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