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GENETIC ANALYSIS OF TYROSINASE GENE AMONG DROMEDARY CAMELS

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ABSTRACT

The Arabian camel, specifically the dromedary camel (*Camelus dromedarius*), has garnered considerable economic interest owing to its remarkable adaptation to desert climates, its valuable byproducts, engagement in racing, and participation in beauty contests. Various phenotypes exhibit distinct economic traits with the tyrosinase gene playing a pivotal role in determining camel coat colors. This study delves into a comprehensive analysis of the complete sequencing of the tyrosinase gene across 16 diverse dromedary camel phenotypes. These phenotypes encompass a spectrum of eumelanin and pheomelanin coat colors, including white (4), black (3), and dark brown (4) diluted including creamy, light brown, and fawn (5). Through sequencing analyses, this study uncovered single nucleotide polymorphisms (SNPs) and deletions within the tyrosinase gene. Notably, white phenotypes exhibited a higher frequency of A↔G (109) nucleotide substitutions, while C↔T (312) mutations were more prevalent across all observed phenotypes. These findings draw valuable insights when higher sample size is analyzed derive the genetic variations associated with coat colors in dromedary camels, shedding light on potential implications for selective breeding programs and furthering our understanding of the intricate genetic mechanisms governing these economically significant traits.

Keywords: Dromedary Camel, Tyrosinase Gene, Coat Color, Single Nucleotide Polymorphisms (SNPs), Traits.

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INTRODUCTION

The dromedary camel is a vital part of Saudi Arabia's cultural heritage and economy. The dromedary camel has been selectively bred over generations, giving rise to distinct phenotypic breeds that reflect the unique adaptability to the Arabian Peninsula (AlAskar *et al.* 2020). This exploration delves into the fascinating world of dromedary camel phenotypic breeds in Saudi Arabia, where the interplay of natural selection and traditional breeding practices has sculpted animals with specific physical traits tailored to the demands of their local habitats and the diverse needs of the communities that depend on them (Alhajeri *et al.* 2021; Giantsis *et al.* 2022).

The tyrosinase gene plays a crucial role in melanin pigmentation in mammals. Melanin is the pigment responsible for the coloration of skin, hair, and eyes in animals, including humans (Anello *et al.* 2019; Nowier *et al.* 2020; Sheikh *et al.* 2021). There are two main types of melanin: eumelanin, which is responsible for black and brown colors, and pheomelanin, which contributes to red and yellow hues.

The tyrosinase gene is a key enzyme involved in the biosynthesis of melanin. It is responsible for catalyzing the rate-limiting steps in the production of melanin from the amino acid tyrosine (Yap & Gan 2021). Variations (polymorphisms) in the tyrosinase gene can influence the activity of the enzyme, affecting the type and amount of melanin produced (Lamoreux *et al.* 2010). Certain mutations may result in reduced or absent tyrosinase activity, leading to conditions such as albinism, where individuals lack normal pigmentation in their skin, hair, and eyes (Dolinska *et al.* 2014; Sun *et al.* 2018). The tyrosinase gene and its role in melanin pigmentation have evolutionary significance and melanin provide protection against harmful ultraviolet (UV) radiation from the sun, and variations in pigmentation have evolved in response to different environmental conditions, such as the intensity of sunlight in various geographic regions (Singh *et al.* 2013). Variations in this gene evolved as the antioxidant defense against environmental stress (Ali *et al.* 2023), useful in the potential molecular marker for breeding specific phenotypes (Jia *et al.* 2021) and these types of data are also useful in the economic trait improvement and data conservation (Xie *et al.* 2024).

There are four basic types of dromedary camels in Saudi Arabia based on their coat color such as black (Majahim), brown (Al Hamar), yellow (Al Sufr) and white (Wodh) (Bitaraf Sani *et al.* 2022; Sheikh *et al.* 2021). However, there are different phenotypic camels in various regions of Saudi Arabia, and they are mainly used in milk, meat production and racing (Table 1).

Table 1. Different phenotypic camels in Saudi Arabia (Abdallah & Faye 2012; Mahmoud *et al.* 2020).

S.No	Coat Color	Local Name
1	Black	Majahim
2	White	Wodh
3	Brownish Yellow	Safrah
4	Yellowish to Red	Hadahana
5	Red to White	Awadi
6	White	Awarik
7	Brown	Hamrah
8	Red	Saheli
9	Grey	Shaele
10	Grey	Shageh
11	Dark Brown	Sofor
12	Blue Grey	Zargeh

The phenotypic appearance and genotypic makeup of the animal plays a crucial role in traditional breeding (Giantsis *et al*., 2022).

There are some other related genes influence the melanin pigmentation, particularly those associated with tyrosinase such as Oculocutaneous albinism 2 (OCA2), Solute Carrier Family 45, Member 2 (SLC45A2) or Melanoma Antigen, Family A (MATP), ASIP agouti signaling protein, proto-oncogene receptor tyrosine kinase (KIT) and melanocyte-stimulating hormone receptor (MC1R) (Fernandes *et al.* 2023; Li *et al.* 2023).

Understanding the molecular processes governed by the tyrosinase gene is crucial not only for unraveling the intricacies of pigmentation in mammals but also for insights into genetic disorders, evolutionary adaptations, and potential applications in fields like dermatology and cosmetic science. This study aims to sequence the whole tyrosinase gene of different dromedary camel phenotypes for polymorphic sites and analyze its role in determining the pigmentation process.

MATERIALS AND METHODS

Animal Ethics: Animal ethics approval was obtained from the Deanship of Scientific Research, Research Ethics Committee, as per the guidelines of King Faisal University with the reference number (KFU-REC/2020 – 09 – 31).

Sample Information: Blood samples were collected from the jugular veins of 16 dromedary camels by authorized veterinarians, ensuring no harm was caused to the animals. The camels represented various coat colors, including white (4), black (3), dark brown (4), and diluted including creamy, light brown, and fawn (5).

DNA Isolation and Polymerase Chain Reaction (PCR): Blood DNA was extracted from the 16 different dromedary camels by using Invitrogen (PureLink® Genomic DNA Kits) as per the manufacturer's instructions. It was quantified on nanodrop (Thermo Scientific™ NanoDrop 2000c spectrophotometer) and confirmed in 1% agarose gel electrophoresis. The extracted DNA was used in PCR (Applied Biosystems, GeneAmp® PCR System 9700). The primers were designed from National Center for Biotechnology Information (NCBI) Primer BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi) and synthesized from Macrogen Inc, Korea. Table 2 shows the primer set used along with annealing temperature and PCR product length. The 25µl PCR mix was prepared with Taq DNA Polymerase 2X Master Mix RED (Denmark), 10 picomoles of forward and reverse primers, quantified DNA (100ng) template and water to make up the total volume. The PCR program was optimized at the following conditions: step 1: Initial denaturation at 94°C - 3min, step 2: denaturation at 94°C - 40sec, annealing at 59.5°C - 50sec, extension at 72°C - 40sec (step 2 repeated for 30 cycles) and step 3: final extension at 72°C - 5min. The amplicons were visualized on agarose gel electrophoresis under UV illuminator. The amplified bands of 2020 bp size were purified and sequenced by Sangers method from Macrogen Inc, Korea.

Sequencing and data analyses: The sequenced samples underwent meticulous analysis using CodonCode Aligner version 9.0.1 (https://www.codoncode.com/aligner/new90.htm). Employing both forward and reverse sequencing, alongwith cross-referencing with the designated reference sequence (XM_010990867.2), a robust consensus sequence was established for each sample. The alignment process adhered to stringent quality standards, ensuring a minimum Phred score of 20 (indicating 99% accuracy). Additionally, chromatograms were manually scrutinized in a systematic manner to authenticate peak accuracy, reducing the likelihood of sequencing errors or ambiguities. The identified contigs (a group of sequences representing similar variations) in the CodonCode program were carefully annotated to highlight SNPs, enhancing the precision and reliability of the obtained genetic data.

Table 2. Primers used in PCR.

S. No	Name	Primers	Annealing Temp	Product length in bp
1	*TYR*	F-TGAAAAGCGAAGTGTGTGGC R-GACGGGCCTCTGTTTTCAGT	60°C	2020

TYR- Tyrosinase gene; F- Forward primer; R-Reverse primer; bp- base pairs

RESULTS

Tyrosinase gene of Dromedary camel: This study conducted a comprehensive sequencing of the tyrosinase gene in dromedary camels, encompassing various coat colors. The gene revealed a predicted coding sequence spanning 1724 base pairs, encoding a tyrosinase protein with 574 amino acids. Remarkably, this protein demonstrated a high degree of conservation, exhibiting 99.67% identity with *Camelus ferus* (XM_006192400.2) and 99.55% with *Camelus bactrianus* (XM_010973375.1). Compared to the NCBI reference sequence XM_010979466.2, revealing 1457 nucleotide variations within coding and non-coding regions across 16 samples. Among these variations, 859 were transversions, 575 were transitions and 23 involved deletions (some of them were represented in Table 3). Detailed information on each sample's complete set of variations offered a valuable resource for a comprehensive understanding of the genetic diversity within the tyrosinase gene in dromedary camels.

Tyrosinase gene Sequencing and polymorphisms

Table 3. Some of the variations found in Tyrosinase gene against reference sequence positions.

C↔G	T↔G	C↔T	G↔A	A↔T	A↔C	Deletion
447C>G	489T>G	660C>T	541G>A	1228A>T	1376A>C	1397G>-
476C>G	1258T>G	665T>C	1484G>A	1378T>A	1482C>A	1398C>-
776G>C	1609G>T	668T>C	1487A>G	1392A>T	1629C>A	
1394C>G	1613G>T	1257C>T	1510G>A	1488A>T	1743C>A	
1506C>G	1696T>G	1492C>T	1607G>A	1605A>T	1832A>C	
1606G>C		1610C>T	1623A>G	1611A>T	1853A>C	
1614G>C		1630C>T	1745G>A	1615A>T		
1683G>C		1635T>C	1836A>G	1626A>T		
1829G>C		1827T>C				
1979G>C		1830C>T				
2014G>C		1833C>T				
		1842T>C				
		1849C>T				
		1959C>T				
		1963C>T				
		1975T>C				
		1976T>C				

Each variation existed in all phenotypes, at least each per phenotypic group.

Characterization of SNPs: The most extensive array of variations was observed within the phenotypic camel group exhibiting diluted coat colors, encompassing creamy, light brown, and fawn hues. Following closely in variation count were the white camel group, succeeded by the black and dark brown phenotypes, as visually depicted in Fig. 1. This distribution highlights the distinctive genetic diversity present in the different coat color categories, with a notable concentration of variations within the diluted phenotypic group. Some of the variations in the sequences showing chromatogram peaks were illustrated in Fig. 2.

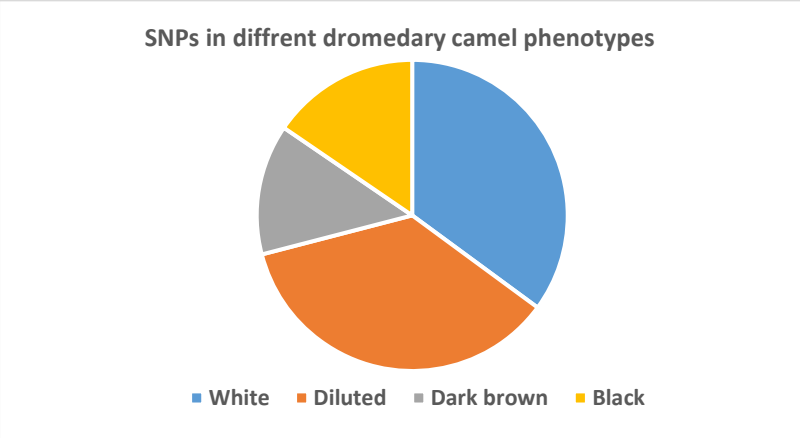

Figure. 1. Graph showing the SNPs contribution from four camel phenotypic groups.

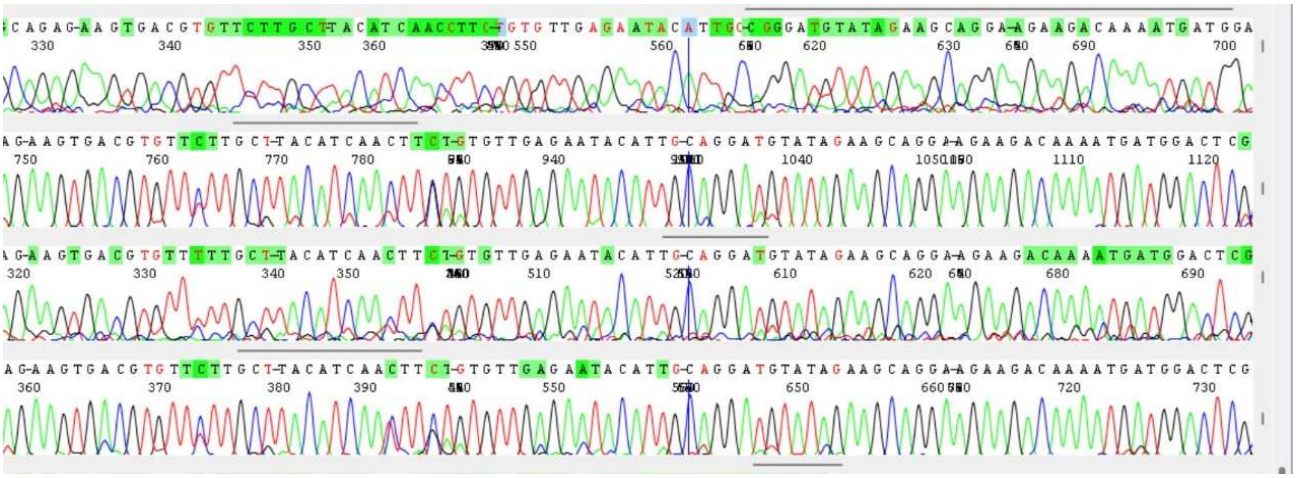

Figure. 2. Tyrosinase gene sequences showing the chromatogram peaks with variations

DISCUSSION

The tyrosinase gene holds paramount significance in the context of genetic studies, particularly concerning the coat colors of dromedary camels. As an essential enzyme in the melanin biosynthesis pathway, tyrosinase plays a pivotal role in determining the pigmentation of hair, skin, and eyes (Wakamatsu & Ito 2023). Sequencing the tyrosinase gene is crucial for understanding the genetic variations responsible for diverse coat colors exhibited by dromedary camels. The sequencing process unravels SNPs within the gene, providing valuable insights into the molecular basis of coat color variations. While previous studies have undertaken partial assessments of SNPs within the tyrosinase gene, the current investigation distinguishes itself by conducting a thorough and comprehensive sequencing of the tyrosinase gene. The primary objective is to discern potential mutations comprehensively, providing a more detailed understanding of the gene's genetic makeup. Remarkably, the tyrosinase gene demonstrates a high degree of conservation, sharing notable similarities with humans and other species. Within the gene, particularly in non-coding DNA regions, the presence of repeated sequences known as microsatellites has been observed. These microsatellites, although residing in non-coding regions, are speculated to play a significant role in gene transcription and may influence phenotype pigmentation with their number of repeats in the intronic region and their length, (Li *et al.* 2004; Sjakste *et al.* 2013). This nuanced exploration sheds light on the intricate molecular mechanisms associated with the tyrosinase gene, contributing valuable insights into its evolutionary conservation and potential functional roles in pigmentation across different species, including implications for the diverse coat colors observed in dromedary camels (Ishag *et al.* 2013; Mahmoud *et al.* 2020; Nowier *et al.* 2020). Certain mutations within the tyrosinase gene have been documented to induce alterations in gene expression, thereby impacting phenotype coloration, as evidenced by the occurrence of albino animals across various species (Anello *et al.* 2019; Deng *et al.* 2009; Hudjashov *et al.* 2013; Ishag *et al.* 2013; Jia *et al.* 2021; Utzeri *et al.* 2021). In mice, missense mutations in seven alleles led to the development of hypopigmentation (Challa *et al.* 2016), while a hypopigmented trait in minks was attributed to the Himalayan allele's presence, marked by a specific SNP (Benkel *et al.* 2009). There is linited information on pigmentation mechanisms in camelids. However, few studies focused on coat color genes such as MC1R, ASIP, TYR and MATP in alpacas. The two SNPs were found correlated to the eumelanin phenotype (Daverio *et al.* 2016). However, these genes (TYR, KIT and MITF) expressed less in white phenotypes (Anello *et al.* 2019; Sheikh *et al.* 2021). In our study, we meticulously examined the tyrosinase gene in camels exhibiting different phenotypes, namely white, diluted, dark brown, and black to identify nucleotide variations. Intriguingly, the white phenotype displayed a higher incidence of variations compared to other groups, a trend corroborated by the findings presented in Table 4. Notably, the A↔G nucleotide substitution was significantly more prevalent in white phenotypes, consistent results from a previous study on llamas (c.428 A>G), suggesting a potential association with phenotype manifestation (Anello *et al.* 2019). Furthermore, the C↔T nucleotide shift exhibited a significant increase in diluted phenotypes, followed by dark brown and black phenotypes, hinting at distinct genetic patterns underlying these diverse coat colorations. There could be number of factors that may influence the coat color such as different genetic background of the rabbit strains with the same tyrosinase SNP resulting in various phenotypes (Aigner *et al.* 2000), Combination of the two gene variants together (TYRc.1205 G > A and OCA2c.1327 G > A) was effective to cause Albinism with heterozygosity while it

was not found with individual variant (Green *et al.* 2024) and SNP in tyrosinase promoter region (SNPc2228A>T) also influence the phenotype pigmentation (Yu *et al.* 2017).

Table 4. Tyrosinase Nucleotide substitutions in various camel phenotypes.

Camel Phenotype	Nucleotide substitutions					P- value	
		A↔G	A↔T	A↔C	G↔C	G↔T	C↔T
White	109	58	73	97	76	98	0.00024
Diluted	96	77	75	80	74	113	0.0004
Dark brown	21	36	31	24	24	52	0.0012
Black	38	31	33	47	25	49	0.0521

The numbers are the SNPs per phenotype, C↔T change was the highest and $p<0.05$ is significant.

The C↔T genotype emerged as the predominant nucleotide substitution across nearly all groups, with its highest prevalence noted in the diluted phenotypes—a pattern consistent with findings in a prior study on llamas (Anello *et al.* 2019). While this C↔T genotype was not exclusive to diluted phenotypes, its abundance across various groups suggests that similar mutations, within diverse genetic environments, might yield distinct genotypes, a phenomenon observed in different species (Benkel *et al.* 2009; Jia *et al.* 2021). It is essential to acknowledge that the C↔T genotype alone may not be the sole determinant of diluted phenotypes, implying the potential involvement of additional mutations influencing the overall coat coloration of individual camels. This nuanced perspective underscores the intricate interplay of genetic factors in shaping phenotypic outcomes. In an evolutionary adaptation perspective, the coat color genes which play a key role in melanin pathway and SNPs specific to the phenotype useful in the better understanding of evolutionary history and phylogenetic relationship (Suzuki 2013; Wu *et al.* 2014). Coat color is important in traditional breeding and selection in farm animals and environmental adaptation (Giantsis *et al.* 2022). It also is in high demand for textiles for fibre (Morante *et al.* 2009) and the loci associated with coat color are useful in selective breeding and purebred selection (Bitaraf Sani *et al.* 2022).

This study analyzed the tyrosinase gene of different dromedary camels for its variations and its association with the different phenotypes. The results were consistent with the previous studies on different species. Two significant genotypes A↔G and C↔T suggests possible association with the coat colors of the dromedary camels. Our data provided the nucleotide sequencing of the tyrosinase gene in the dromedary phenotypes which are useful in understanding the molecular units variations of the different camel phenotypes and their classification. This knowledge not only enhances our comprehension of the intricate genetic mechanisms governing pigmentation but also holds practical implications for selective breeding programs when used larger sample data of diverse camel breeds along with other related genes in Saudi Arabia, contributing to the development of camel phenotypes with specific economic traits.

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Authors' Contribution: AS did the Conceptualization, methodology, analysis, writing - original draft preparation.

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