Helalia *et al.*
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RESEARCH

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Unveiling polymorphism and protein structure prediction insights in diacylglycerol O-acyltransferase 1 and telethonin genes of Egyptian buffalo

Sahar M. Helalia[1], Aida I. El Makawy[1*], Neama I. Ali[1], Nagwa H. A. Hassan[2] and Mohamed S. Hassanane[1]

Abstract

Background The Egyptian buffalo has a sizable impact on Egypt's agricultural sector and food supply. It is regarded as the main dairy animal and an important source of red meat. This study aimed to detect the polymorphisms of the *DGAT1* and *TCAP* genes and assess the potential impact of the discovered nsSNPs on the stability of the tertiary structure polypeptides of selected genes in Egyptian buffalo.

Methods Allele identification was made by the restriction fragment length polymorphism (RFLP), and the single nucleotide polymorphisms (SNPs) were recognized by sequencing the purified PCR products. Protein translation indicated the synonymous and non-synonymous SNPs, and the peptides' 3D tertiary structure of selected genes, as well as the effect of amino acid substitution on the protein structure, was performed using bioinformatics tools.

Results Analysis of the data revealed that an nsSNP was detected in a tested region of the *DGAT1* gene and caused an amino acid substitution in a polypeptide that was predicted to be neutral and located in the coiled part of the protein. The analysis of the *TCAP* gene showed four nsSNPs that caused four substitutions located in the α-helix region. Protein prediction analysis showed that the amino acid substitutions in *DGAT1* and *TCAP* were non-conserved with low sensitivity to variation. The non-conservative amino acid substitutions result in amino acids with new properties different from the original amino acid that change the protein's structure and function.

Conclusion We can infer that the *DGAT1* and *TCAP* genes' SNPs may affect meat-related traits and may improve meat quality.

Keywords Functional genes, Buffalo, Polymorphism, Restriction fragment length polymorphism, Protein prediction

Background

Water buffaloes are imperative resources for the nourishment of global humans, as they provide meat and very nutritious milk. Breed-specific genome records and sequencing are not yet available. Furthermore, there is a lack of knowledge regarding economically significant characteristics, particularly the production and quality of meat (El Debaky et al., 2019).

Genome biotechnology by manipulating intra- and interbreed genetic diversity offers prospects for improving sustainable animal production systems. Genomic

*Correspondence:
Aida I. El Makawy
aelmakawy@yahoo.com
[1] Cell Biology Department, Biotechnology Research Institute, National Research Centre, 33 El Bohouth St., Dokki, P.O. 12622, Giza, Egypt
[2] Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt

characterization is required for phenotypic differentiation, mate selection, and producing good offspring. The development of a comprehensively annotated and assembled reference genome for buffaloes is necessary due to the importance of buffaloes as a genetic resource on a global scale (Rehman et al., 2021).

Next-generation sequencing (NGS) technology was used to produce a large number of DNA fragments covering the genome with a respectable depth suitable for the assembly to get around these challenges (El-Khishin et al., 2020). NGS is a vital tool for metagenomic inquiry and detecting, controlling, and monitoring infectious illnesses (Berry, et al., 2020).

The *DGAT1* and *TCAP* genes were selected based on their essential association with the traits of meat and milk quality in buffalo, cattle, or other allied species. From a genetic standpoint, determining the genotypic distribution of markers linked to economically important traits may be a powerful tool for gaining immediate knowledge of livestock breeds and populations' productive potential (Marshall et al., 2019). *DGAT1* and *TCAP* have been recognized as functional genes during fat deposition and are closely related to meat quality (Gao et al., 2020). *DGAT1* and *DGAT2* are key enzymes for catalyzing the terminal step in the formation of trigylecrols (TGs) through the acylation of diacylglycerol (DAG) with a fatty acyl-CoA, thus regulating lipid digestion, absorption, and glycerol lipid metabolism pathways (Bhatt-Wessel et al., 2018). It is situated on bovine chromosome 14, spans 14,117 bp, and comprises 17 exons. The substitution named ApA to GpC dinucleotide is found in exon VIII of the bovine *DGAT1* gene, which changes lysine K to alanine A in the encoded protein (K232A polymorphism) and has a pronounced influence on milk yield and composition, especially on the fat percentage in milk (Li et al., 2021). Besides *DGAT1*'s association with milk production traits, it was discovered to be a gene associated with meat production in a variety of animals, including buffalo (Khan et al., 2021; Urbinati et al., 2016).

Understanding the potential genes governing skeletal muscle development is therefore essential for comprehending the molecular genetic control of muscle growth and can help the meat industry achieve its objective of increasing meat yields (Mohammadabadi et al., 2021). *TCAP* (titin-cap or telethonin) is one of the titin-interacting Z-disk proteins that controls and promotes the normal growth of sarcomeric structure (Qiao et al., 2014). *TCAP* has been shown to interact with other proteins that influence cell growth and differentiation: the potassium channel B subunit mink, Ankrd2, murine double minute 2 (MDM2), and protein kinase D (Haworth et al., 2004; Kojic et al., 2004; Tian et al., 2006). Previous work demonstrated the high potential of *TCAP* as a marker gene for the development of cattle or other animals for growth performance and carcass quality traits (He et al., 2018).

This study's goal was to use the restriction fragment length polymorphism technique and sequencing to find single nucleotide polymorphisms in the *DGAT1* and *TCAP* genes in selected areas of Egyptian buffalo individuals. Bioinformatics techniques were utilized to further investigate how the discovered SNPs affect protein structures and functions.

Methods

Collecting of blood samples

The blood samples used in this study were collected from 84 male buffaloes by veterinarians through routine blood specimens from a farm in Kafr Almayasrah, Damietta Governorate, Egypt. The blood sampling was done specifically for this study, and the animals were not linked to any experimental design. Blood samples were collected from the jugular vein using 15-ml sterile test tubes containing anticoagulant (EDTA) and then stored at −20 °C until DNA isolation.

Molecular methods

DNA isolation

Centrifugation was utilized to separate the white blood cells from the blood, and the salting out method was applied to extract genomic DNA from all of the collected blood samples according to (Suguna et al., 2014) with some modification, and then its purity and concentration were detected by the NanoDrop 1000 analyzer (Thermo Fisher Scientific, Wilmington). DNA was then diluted to the working concentration of 50 ng for the polymerase chain reaction (PCR) (Garcia-Alegria et al., 2020).

PCR, genotyping, and sequencing

PCR amplification was performed in a 20 µL reaction mixture containing 2 µL genomic DNA, 2 µl of 10X PCR buffer, 1 µl of each forward and reverse primer (10 µM), 2 µl dNTPs (10 mM), 0.1 µl (0.5 unit) of Taq DNA polymerase, and 11.9 µl ddH2O to make up the volume. The primer sequences for *DGAT1*: F-GCACCATCCTCT TCCTCAAG; R-GGAAGCGCTTTCGGATG (Thaller et al., 2003) and *TCAP*: F-GGGAGTGAGCAGTCATCA TGGC; R-AGAGGCAGCACCCGCTGGT (Cheong et al., 2007). The *DAGT1* and *TCAP* gene loci were examined for polymorphisms using the RFLP technique. Thermo Scientific's *EaeI* and *BtsCI* fast digest restriction enzymes were used to digest 10 µL of the PCR products for an hour at 37 °C and then heated to 65 °C for 5 min to deactivate the restriction enzymes. On a 2% agarose gel stained with ethidium bromide (GIBCO, BRL, England) and running in 1X TBE buffer, the restriction fragments

were electrophoresed. The gels were visualized under UV and photographed using the Gel documentation system (Bio-Rad Laboratories Inc., USA).

PCR cleaning and sequencing

The PCR products displaying different RFLP banding patterns were selected for sequencing. Each genotype of the chosen genes' PCR products was purified and sequenced by Macrogen Incorporation (Seoul, Korea). Codon Code Aligner software from the Codon Code Corporation (USA) was used to perform the sequences and alignment exploration.

Data analysis

DnaSP 5.00 software was used to determine haplotype structure, sequence variation, average number of nucleotide differences, and substitutions per site between samples. Mega version 11 software was used to create the genetic analysis (Kumar et al., 2016). *DGAT1* and *TCAP* gene sequences were analyzed and aligned using NCBI/BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Homolog sequences were downloaded in FASTA format from the NCBI GenBank database and aligned with BioEdit (v7.0.9). The EXPASY program was used to predict the protein sequences. The amino acid substitutions brought on by the SNPs were discovered, and non-synonymous SNPs (nsSNPs) were detected. The impact of the nsSNPs on protein activities was predicted using the web-based tool PredictSNP (Bendle et al., 2014). Additionally, protein homology analogy recognition engine (Phyre2) software was used to evaluate protein 3D tertiary structure (Kelley et al., 2015).

Results

In the current study, the genetic polymorphisms of *DGAT1* and *TCAP* were screened in Egyptian buffalo via PCR–RFLP and DNA sequencing for SNP detection.

Diacylglycerol O-acyltransferase (*DGAT1*) gene

The PCR amplification of the *DGAT1* gene resulted in a fragment of 413 bp, which extended from exons 7–9 in the tested sample of Egyptian river buffalo. The digestion of the *DGAT1* gene amplicon with restriction endonuclease *Eael* revealed a single undigested band, indicating that there was no polymorphic region, implying that all buffalo animals were homozygous for the AA allele (Fig. 1A). The *DGAT1* sequence alignment displayed two haplotypes, synonymous C73G and non-synonymous C74G SNPs (Fig. 1B).

Protein analysis of the *DGAT1* gene clarified that the 413 bp of the gene were converted to 133 amino acids with nsSNP C74G, which caused a change from amino acid glutamine (Q) to glutamic acid (E) at position 25, resulting in a Q 25 E substitution in the *DGAT1* polypeptide. The 3D tertiary structure of the Egyptian river buffalo *DGAT1* peptide showed that the model was fitted with 99.7% confidence by the single highest-scoring template and 105 amino acid residues that covered 79% of the sequence (Fig. 2A). The predicted secondary structure in the 3D model of the *DGAT1* protein consists of the α-helix structure representing 20% of the model, beta

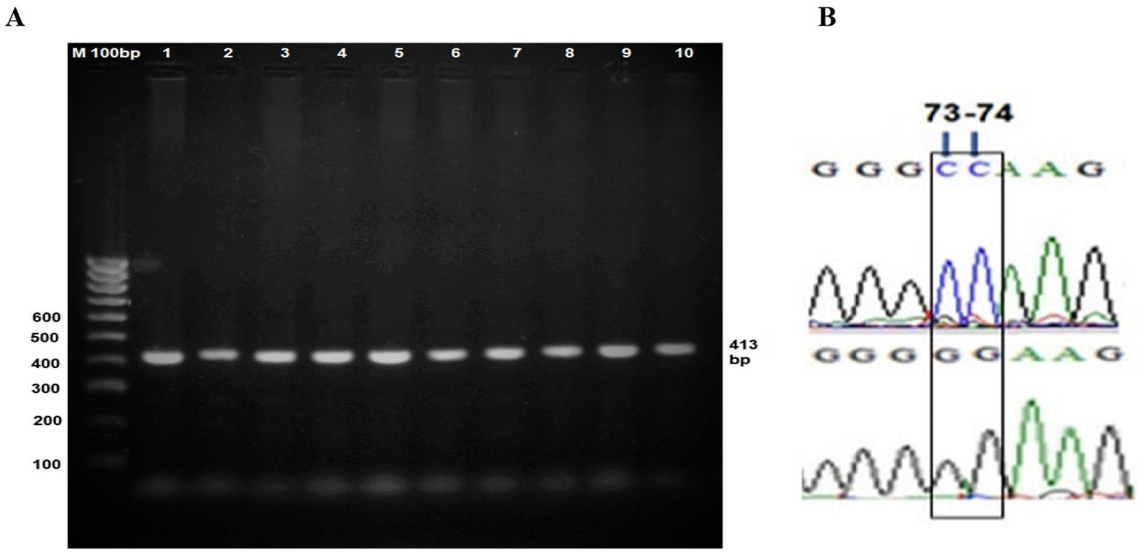

Fig. 1 **A** Electrophoretic pattern obtained after treatment of PCR amplified DGAT1 locus with Eael restriction enzyme; Lane M: 100 bp DNA marker, Lanes (1–10): undigested band at 413 bp representing AA genotype; **B** Chromatograms showing C/G SNPs

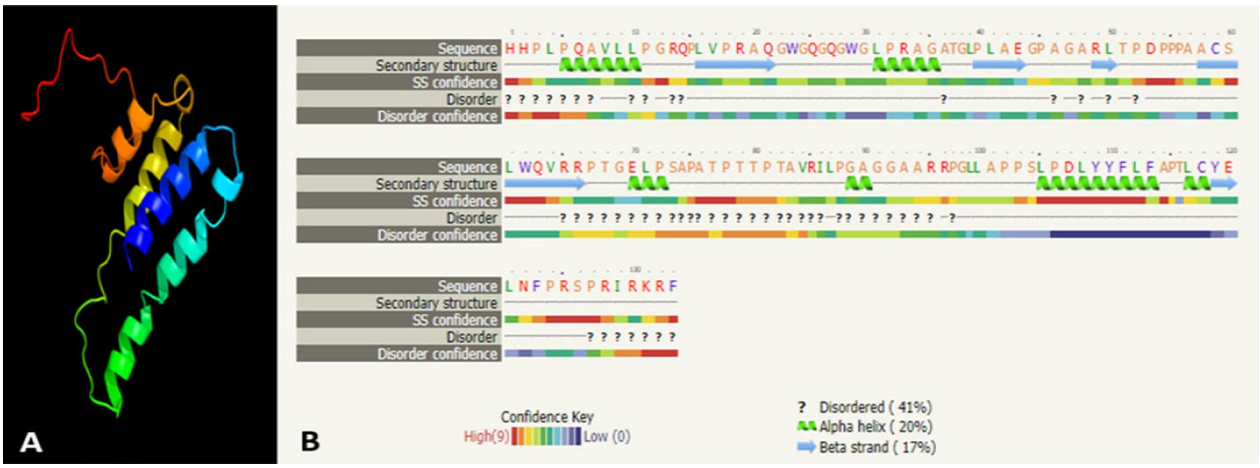

Fig. 2 Egyptian water buffalo DGAT1 **A** the predicted 3D tertiary structure of polypeptide; **B** Secondary structure of the 3D model of protein consists of α-helix (green), beta strands (blue) and disordered regions (?). The "SS confidence" line displays the prediction's level of confidence, with red denoting a high level and blue denoting a low level

strands representing 17%, disordered regions representing 41% of the model, and pale colors showing the coiled regions (Fig. 2B).

The assessment of the potential impact of an amino acid substitution due to non-synonymous SNPs in the *DGAT1* gene on the structure and function of proteins using PredictSNP revealed that the Q25E SNP was predicted to be neutral (Fig. 3A). Protein prediction of amino acid substitution Q25E showed that the wild amino acid glutamine is a low-conservative amino acid located in a coiled region with low sensitivity variation (Fig. 3B).

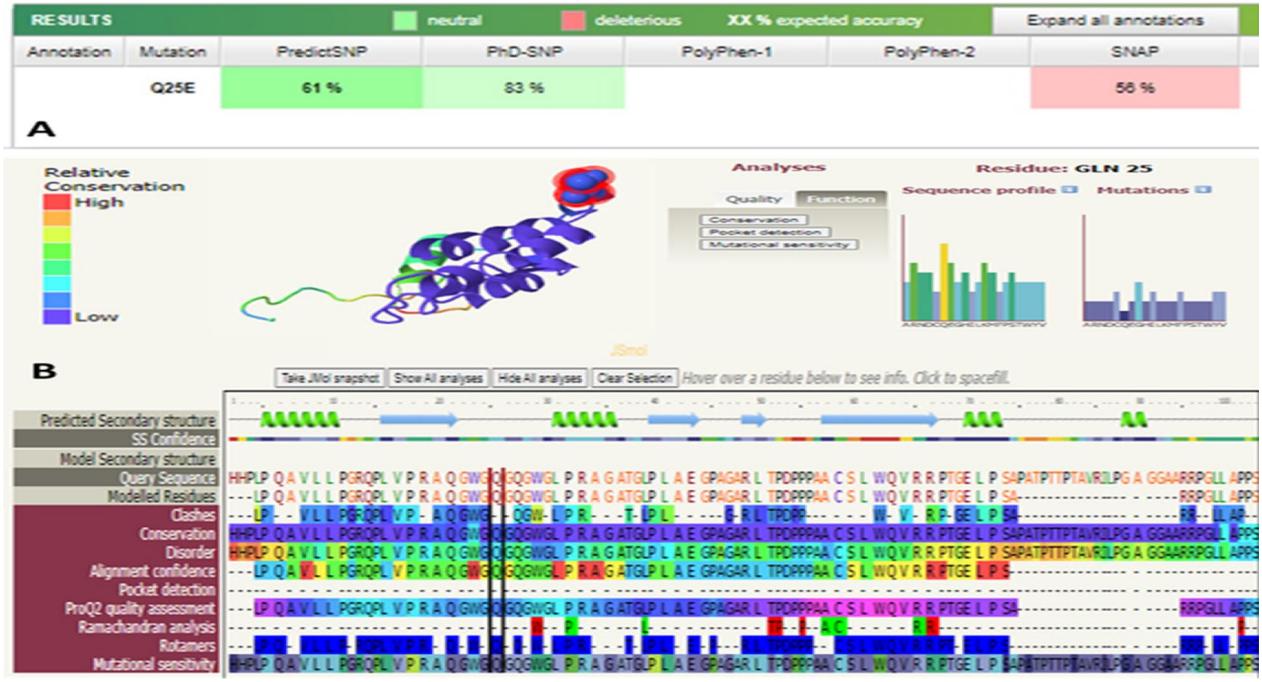

Fig. 3 **A** Analysis of DGAT1 amino acid nsSNPs using PredictSNP Software. **B** Phyre2 investigator result showed the wild amino acid glutamine located in a coiled region

Titin-cap/telethonin (*TCAP*) gene

The *TCAP* gene amplicon (517 bp) (Fig. 4A) digestion with BtsCI showed no polymorphic regions; only a monomorphic restriction pattern GG genotype at 306, 152, and 59 bp was found in all of the samples (Fig. 4B). The *TCAP* gene alignment showed four haplotypes that identified six SNPs in exon 1, two of which are synonymous SNPs (T218C and T230C), and the other four are non-synonymous: G165C, T306C, C321T, and C401G (Fig. 5). The four nsSNP caused four substitutions: from glutamic acid to glutamine at position 53 (E53Q), from tryptophan to arginine at position 98 (W98R), from leucine to Phenylalanine at position 103 (L103F), and from histidine to glutamine at position 129 (H129Q), respectively.

The *TCAP* amino acid sequence was aligned with the NCBI database using the cluster-w software (https://www.genome.jp/tools-bin/clustalw). The 517 bp translated into 167 amino acids with four substituted amino acids: glutamic acid to glutamine (E53Q), tryptophan to arginine (W98R), leucine to phenylalanine (L103F), and histidine to glutamine (H129Q). The 3D tertiary structure of the river buffalo *TCAP* polypeptide was predicted using the Phyre2 software. The result showed that the aligned modeled residues were 89 amino acids, covering 53% of the protein sequence with a percent confidence of 100% (Fig. 6A). The predicted secondary structure in the 3D model of the *TCAP* protein consists of the α-helix structure representing 41% of the model; beta strands representing 14%, while the disordered regions representing 48% of the model (Fig. 6B).

The effect of non-synonymous G165C, T306C, and C401G SNPs in the *TCAP* gene on protein structure was predicted using the sequence prediction tool PredictSNP

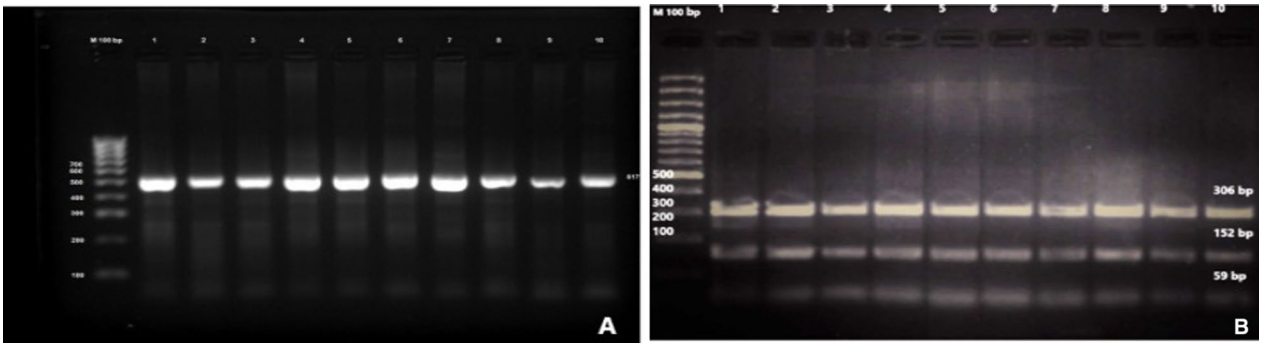

Fig. 4 The TCAP gene PCR products **A** Before digestion; **B** after amplifying with allele-specific primers and cutting with BtsCI restriction enzyme. Lane M: 100 bp DNA marker, lanes (1–7) genotype GG (306, 152 and 59 bp)

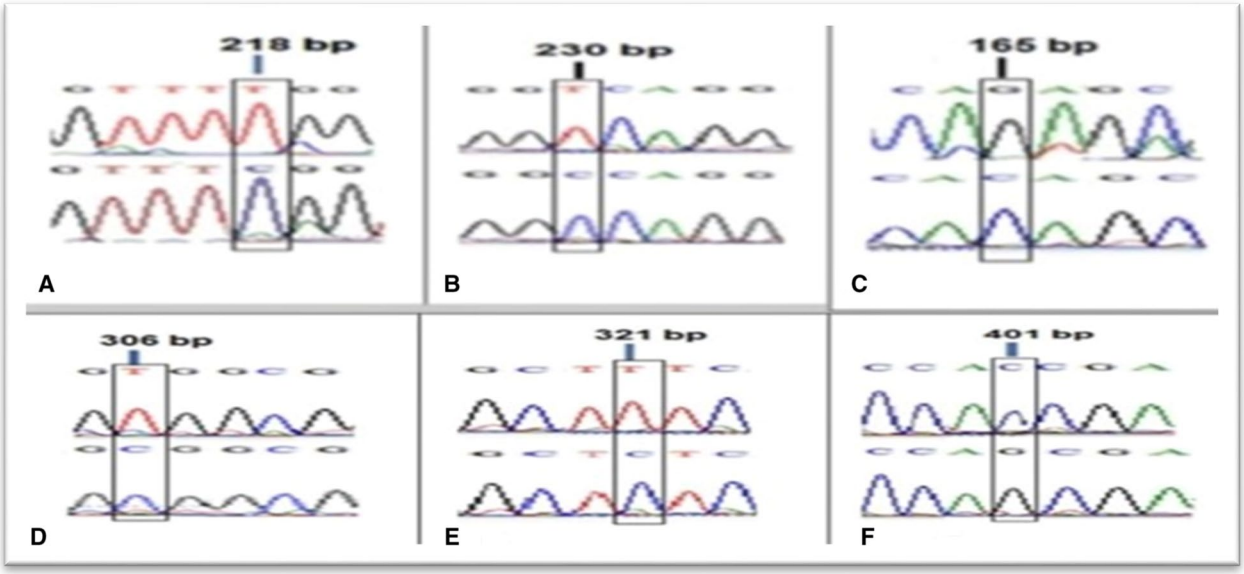

Fig. 5 Chromatograms showing **A**, **B** Synonymous SNPs and **C**–**F** non-synonymous SNPs in TCAP gene

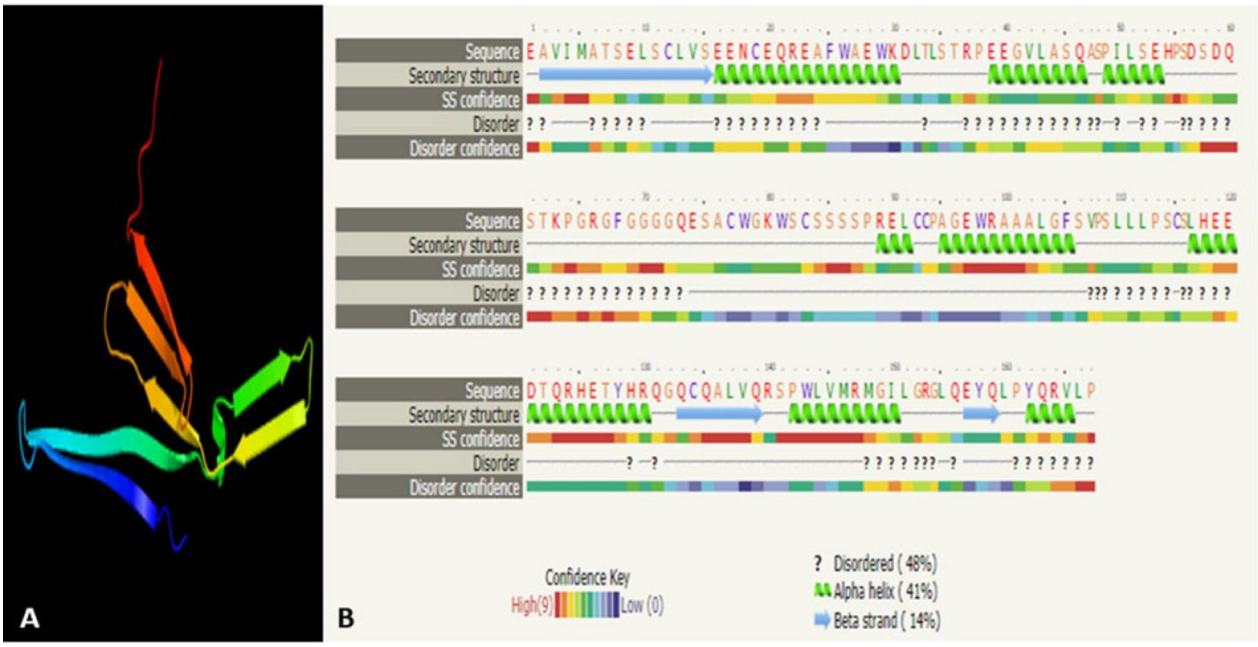

Fig. 6 Egyptian water buffalo TCAP **A** the predicted 3D tertiary structure of polypeptide; **B** Secondary structure of the 3D model of TCAP protein consists of α-helix (green), beta strands (blue) and disordered regions (?)

software. The analysis displayed that the SNP E53Q was predicted to be neutral, while the W98R, L103F, and H1129Q SNPs were predicted to be deleterious (Fig. 7).

E53Q, W98R, and L103F amino acid substitutions showed that the wild glutamic, tryptophan, and leucine amino acids are low-conservative and located in the α-helix region with low variation sensitivity (Figs. 8, 9, 10). While H129Q amino acid substitution showed that the wild amino acid histidine is a semi-conservative amino acid located in the α- helix region with low variation sensitivity (Fig. 11).

Discussion

DGAT1 has brought a great deal of attention to the production of animal milk and meat (Khan et al., 2021). *TCAP* also codes for a protein found in striated muscle and is involved in the quality assessment of meat (Gao et al., 2022). In the present study, the genetic polymorphism of the diacylglycerol O-acyltransferase 1 (*DGAT1*) and Titin-cap/telethonin (*TCAP*) genes was evaluated in the Egyptian buffalo breed using PCR–RFLP analyses to identify the different genotypes of each gene. The *DGAT1* gene is regarded as a crucial enzyme that controls the synthesis of Triacylglycerol (TAG) in adipose tissue (Chitraju et al., 2019). Earlier studies confirmed that polymorphisms in *DGAT1* were significantly associated with milk production traits in water buffalo (Gautier et al., 2007; Spelman et al., 2002; Weller et al., 2003). In addition, the impact of the *DGAT1* gene on traits associated with meat production in cattle has been the subject of numerous studies. It has been established that the *DGAT1* gene might have an impact on the hue and fat content of beef (Ardicli et al., 2018). The *DGAT1* gene can utilize as a genetic indicator to increase milk output in dairy cattle and to aid in the improvement of meat quality and carcass fatness in cattle (Khan et al., 2021).

RESULTS — neutral — deleterious — XX % expected accuracy — Expand all annotations

Annotation	Mutation	PredictSNP	PhD-SNP	PolyPhen-1	PolyPhen-2	SIFT	SNAP
	E53Q	75 %	83 %	67 %	68 %	53 %	56 %
	W98R	55 %	73 %	67 %	71 %	79 %	89 %
	L103F	51 %	83 %	-	-	46 %	72 %
	H129Q	64 %	66 %	59 %	47 %	53 %	62 %

Fig. 7 Analysis of TCAP amino acid nsSNPs using PredictSNP tools

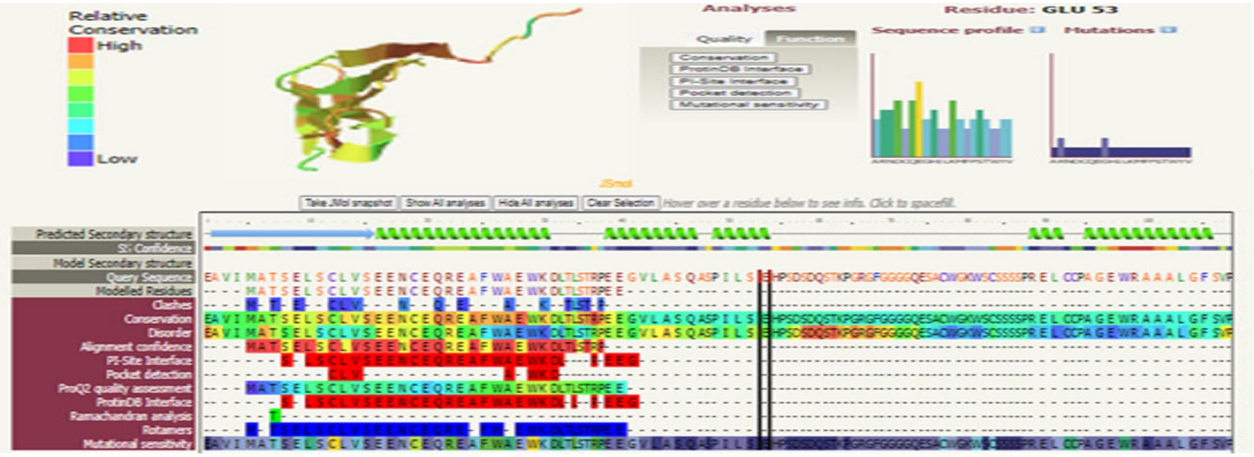

Fig. 8 The result of phyre2 investigator showed the wild amino acid glutamine located in α-helix region

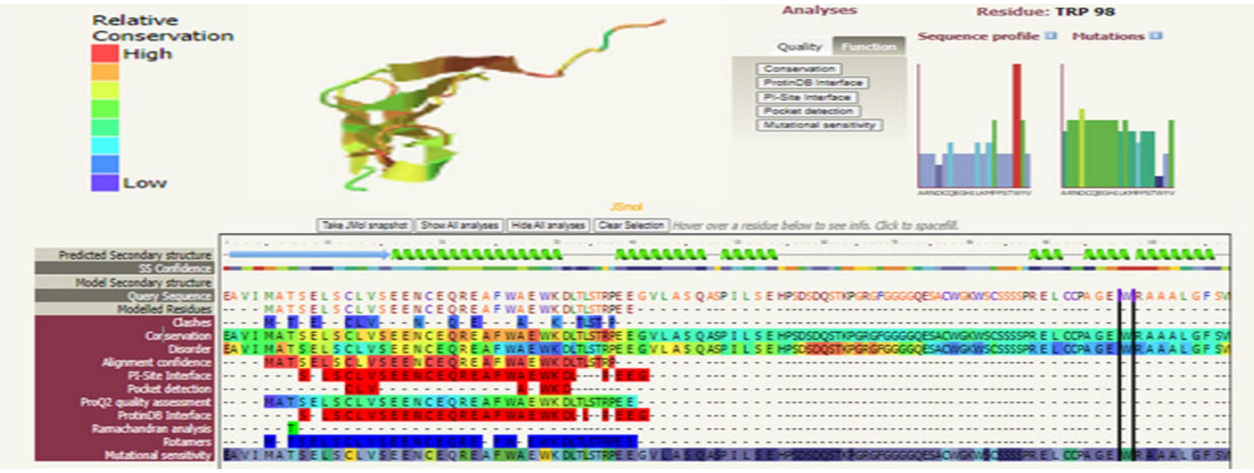

Fig. 9 The result of phyre2 investigator showed the wild amino acid tryptophan located in α- helix region

Fig. 10 The result of phyre2 investigator showed the wild amino acid leucine located in α-helix region

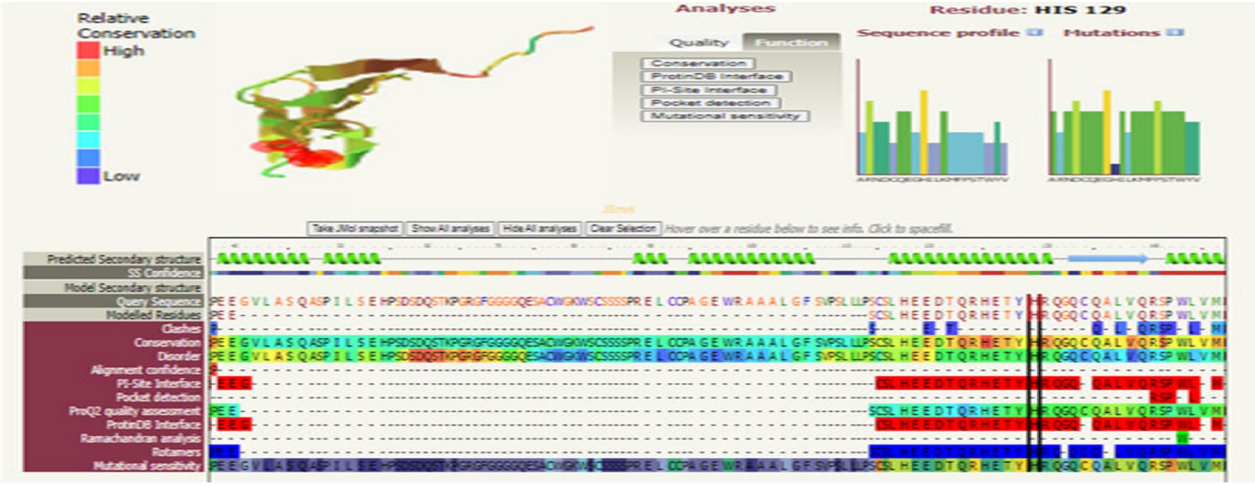

Fig. 11 The result of phyre2 investigator showed the wild amino acid histidine located in α- helix region

Also, based on marker-assisted selection, an earlier study found that some variations in the *DGAT1* gene enhanced the water buffalo's reproduction, growth, milk yield, and composition traits (Isik et al., 2022). Our RFLP analysis for *DGAT1* using the *Eael* restriction enzyme generated an identical pattern in all samples of Egyptian river buffalo with homozygous AA alleles. In agreement with these findings, the AA allele was found in Iranian buffalo, in many Bos indicus breeds like Hariana, Tharparkar, Sahiwal, and Nellore, in Murrah**,** and river Egyptian buffalo (Aboelenin et al., 2017; Heydarian et al., 2014; Silva et al., 2016; Venkatachalapathy et al., 2014). Formerly, three different genotypes (AA, GA, and GG) in Polish Holstein young bulls were identified, and an association between polymorphisms in the *DGAT* gene and meat quality was found (Urtnowski et al., 2011). The *DGAT1* sequence alignment displayed nsSNP C74G that changed the sequence of amino acid glutamine to glutamic at position 25, which affected the protein structure and could motivate the function because the glutamine is non-conserved. In *DGAT1* of Murrah buffaloes, there were three SNPs associated with fat and protein percentages. The detected amino acid substitution (A484V) could stimulate the function of the diacylglycerol O-acyltransferase1 protein and affect milk production and quality traits (de Freitas et al., 2016). Also, G 219A SNP polymorphisms in *DGAT1* of Iranian goats caused the substitution of serine to glycine which might affect the protein structure (Evrigh et al., 2018). Other studies confirmed that *DGAT1* polymorphisms have pleiotropic effects on meat production traits in Polish Holstein bulls, dairy cattle, cows, and beef cattle (Ardicli et al., 2018; Sorbolini et al., 2015; Urbinati et al., 2016; Urtnowski et al., 2011). In addition, the SNPs C > T and T > G substitutions in exon 17 of *DGAT* caused the substitution of threonine to alanine and valine to glycine, which were associated with better meat quality traits in Chinese cattle (Yuan et al., 2013). The SNP (g.9046 T > C) caused amino acid substitution from arginine to histidine in exon 17 of the *DGAT1* gene, and was associated with the fat percentage in river buffalo and swamp buffalo (Li et al., 2018).

The protein *TCAP* is a myofibrillar protein that plays an important role in the assembly of myofibrils and is crucial for muscle growth and fat deposition (Olive et al., 2008). Previous research suggests that the *TCAP* gene plays a critical role in muscle differentiation and regeneration during muscle development in vitro and in vivo (He et al., 2018). The amplified DNA fragment of 517 bp from buffalo *TCAP* gene digestion with *BtsCI* showed three fragments of 306, 152, and 59 bp for allele G with genotype GG, and there was no polymorphism, indicating the homozygosity of this gene. Contrariwise, a former investigation reported that the digestion of the amplified DNA fragment of *TCAP* (517) in Nellore cattle with *BtsCI* produced fragments of 177, 154, 128, and 58 bp for allele A and 305, 154, and 58 bp for allele G (Borges et al., 2014). The gene alignment showed four haplotypes that identified six SNPs, two of which are synonymous (T218C and T230C), and the other four are non-synonymous (G165C, T306C, C321T, and C401G). Previous research mentioned that the *TCAP* was not polymorphic for g.346G > A in *indicus* cattle. Another research team discovered four single nucleotide variants (SNVs) in almost all of the exon and intron regions of the *TCAP* gene of cattle that were significantly associated with the growth performance and carcass quality traits of Qinchuan cattle (He et al., 2018). Our study showed that the coding sequence of *TCAP*, comprising 167 amino acids, shares

high sequence similarities with Bubalus bubalis (99%). Meanwhile, the full-length coding sequence of the bovine *TCAP* gene, comprising 166 amino acids showed high sequence similarities with the human (95.8%) and mouse (95.2%) *TCAP* genes (Yu et al., 2004). However, to date, no polymorphisms have ever been reported in *TCAP* in farm animals.

The quality of the meat is influenced by a variety of factors, including the animal's breed, nutrition, feeding method, and age. Meat contains a wide range of proteins, carbohydrates, lipids, and other nutrients. Amino acids are essential nutrients that contribute significantly to the taste and flavor of the meat. Amino acids are not only necessary components of proteins, but they also influence the synthesis of other muscle components (Ma et al., 2020). One of the best indicators of how missense variants will affect phenotype-structural information in proteins. They can change functional residues or destabilize the entire protein fold, which affects protein structure and function (Stefl et al., 2013). In the current study, assessments of the potential impact of the discovered nsSNPs on the stability of the tertiary structure of selected genes polypeptides revealed that nsSNP Q25E in the *DGAT1* gene showed that the wild amino acid glutamine is non-conserved and is located in the coiled region. Besides, the amino acid substitutions E53Q, W98R, L103F, and H1129Q evaluated in the *TCAP* gene clarified that glutamic, tryptophan, leucine, and histidine were located in the α-helix region and were seen as non-conserved and impacting the protein structure or function. Both deleterious and neutral missense variations were stated to be mainly situated in helices and coil regions and rarely in β-strands, where β-strands are more intolerant to variations than α-helices (Abrusán & Marsh, 2016; Kucukkal et al., 2015).

Conclusions

Through the sequencing of the Egyptian buffalo's *TCAP* and *DGAT1* genes, several non-synonymous SNPs were found in this study. The identified polymorphisms result in non-conservative amino acid substitutions that alter the structure and function of the protein. In light of the alteration of protein structure and function, we can therefore conclude that the polymorphisms in the Egyptian buffalo's *DGAT1* and *TCAP* genes may improve the traits of meat quality. It is advised to conduct additional genetic characterization of genetic variations in the *DGAT1* and *TCAP* genes by rearing buffalo in various Egyptian regions. This additional genetic characterization could be used in selective breeding systems aimed at enhancing the meat quality of this breed.

Abbreviations

A	Alanine
BLAST	Basic Local Alignment Search Tool
BLASTn	Nucleotide BLAST
bp	Base pair
DGAT1	Diacylglycerol O-acyltransferase 1
ddH2O	Double-distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
E	Glutamic
EDTA	Ethylene diamine tetra acetic acid
E	Glutamic
F	Phenylalanine
H	Histidine
L	Leucine
nsSNPs	Non-synonymous single nucleotide polymorphisms
PCR	Polymerase chain reaction
Phyre2	Protein homology analogy recognition engine
Q	Glutamine
R	Arginine
RFLP	Restriction Fragment Length Polymorphism
Rpm	Revolution per minute
SDS	Sodium dodecyl sulfate
SNP	Single nucleotides polymorphism
Taq DNA	Thermus aquaticus DNA
TBE	Tris–borate-EDTA buffer
TCAP	Telethonin or Titin-cap
TE	Tris–EDTA
W	Tryptophan

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Author contributions
This manuscript was done in collaboration with all authors. AIE and MSH conceived the idea and designed the experiments. SMH performed the experiments; AIE and SMH analyzed the data; AIM and NIA co-wrote the paper; NHAH revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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