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Genetic Diversity of GPAM, DGAT1, and SCD1 Lipogenic Candidate Genes In Egyptian and Indian Buffalo

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Abstract

THE objectives of the present study were to analyze polymorphisms in the glycerol-3-phosphate acyltransferase mitochondrial (GPAM), diacylglycerol O-acyltransferase 1 (DGAT1), and stearoyl-coenzyme A desaturase 1 (SCD1) genes in the Egyptian buffalo breed, and also to explore genetic diversity across Egyptian, Murrah, and Bhadawari buffalo populations. Genotyping was conducted using PCR-SSCP and sequencing techniques. Results indicate significant deviations from Hardy–Weinberg equilibrium (HWE) in the Egyptian buffalo breed for GPAM, DGAT1, and SCD1 genes, with Murrah significantly differing at DGAT1 and SCD1 genes. Bhadawari breed significantly deviated from HWE only at DGAT1 gene. Pairwise comparison between populations revealed the shortest genetic distance between Egyptian and Murrah, while the longest between Bhadawari and Murrah. Multiple SNPs were identified in the genes under study, with alterations of amino acid sequences. These findings reveal novel genetic variants with potential implications for lipid metabolism and milk quality in Egyptian buffalo. Further research is warranted to elucidate the functional significance and breeding applications of these variations.

Keywords: River buffalo, GPAM, DGAT1, SCD1, PCR-SSCP, SNPs, Genotyping.

Introduction

Since their first domestication around 5,000 years ago [1], water buffalo stand as one of the most important agricultural animals, playing a major role in the economy of many developing countries worldwide. Buffalo are crucial for the global agriculture, considerably contributing to the production of milk, meat, skin, and draught power [2]. India and Egypt are among the largest producers of buffalo milk, providing approximately 231,740 and 5,123 million tonnes, respectively, of the global milk production [3].

The Egyptian buffalo breed belongs to the river buffalo (*Bubalus bubalis*) and is regarded as the primary dairy animal in Egypt [4]. Compared to cattle, buffalo is better adapted to poor dietary fiber and protein, exhibiting high feed conversion efficiency on low-quality diets, as well as more resistant to common diseases [5]. Among the wide variety of buffalo breeds recognized in India, Murrah buffalo is the most productive dairy buffalo breed worldwide and renowned for its adaptation to harsh climatic conditions, considerable feed conversion and reproductive efficiency [6]. On the other hand, Bhadawari breed is recognized for having the highest milk fat percentage (12.8% on average) among all buffalo breeds [7], and adaptability to extensive production systems [8]. Given these valuable characteristics, it is essential to understand the genetic factors underlying performance variation observed between and within buffalo breeds.

Genetic characterization and diversity studies are crucial for preserving buffalo genetic resources and enhancing breeding programs [6, 9, 10, 11]. In this regard, SSCP technique is one of the simplest methods for detecting polymorphisms based on PCR. It can detect unknown mutations and is rapid, cost-effective, and convenient technique for SNP

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identification. Several improvements and modifications have been made to improve the differential capacity between the different migrations of single-stranded DNA, thereby increasing the sensitivity of PCR-SSCP for detecting mutations and polymorphisms [12, 13].

Previous research has identified several genes associated with genetic variations in milk fat content [8, 14, 15]. Notably, mutations in glycerol-3-phosphate acyltransferase mitochondrial (GPAM), diacylglycerol O-acyltransferase 1 (DGAT1), and stearoyl-coenzyme A desaturase 1 (SCD1) genes have emerged as potential molecular markers for lipid metabolism and milk fat content in buffalo breeding [16, 17, 18]. GPAM gene, located on chromosome 23 in buffalo [19], is involved in triglyceride and phospholipid biosynthesis in buffalo milk [20]. Variation in this gene has been linked to differences in triglyceride production and lipid metabolism [19]. Importantly, DGAT1 gene that is located on buffalo chromosome 15 [18], encodes the enzyme acyl CoA: diacylglycerol-acyltransferase, which is essential for triglyceride synthesis [21], and has been associated with milk production traits [22, 23]. SCD1 gene, located on buffalo chromosome 23 [16], is essential for converting saturated fatty acids into monounsaturated fatty acids [24]. Previous studies have indicated a noteworthy correlation between polymorphisms in SCD1 gene and milk production traits in dairy cattle. In particular, milk and protein yield in Italian Holstein cows [25], as well as milk and fat content, and protein yield in the Chinese Holstein cows have all been linked to the g.10329C>T polymorphism [26].

Given the valuable importance of genetic factors influencing milk fat content in buffalo populations, the present study aimed at investigating the genetic diversity and polymorphisms in key lipid biosynthesis genes (GPAM, DGAT1, and SCD1) among the Egyptian, Murrah, and Bhadawari buffalo breeds. The study applied PCR-SSCP technique to assess genetic diversity and further characterized polymorphisms within the Egyptian buffalo through the direct sequencing method.

Material and Methods

Animal samples and DNA extraction

This study was approved by the Institutional Animal Care and Use Committee (CU-IACUC) of Cairo University, Egypt (approval number: CU-II-F-45-22). Sample collections was carried out in accordance with the approved guidelines of CU-IACUC. A total of 240 blood samples were collected, comprising 140 Egyptian buffalo, 60 Indian Murrah, and 40 Indian Bhadawari buffalo. Different farms located in the Nile Delta of Egypt contributed to sampling process. Regarding the Indian buffalo breeds, Murrah and Bhadawari were sampled from Haryana and Uttar Pradesh, respectively. Blood (10 ml) was obtained from the jugular vein of the animals by professional veterinarians, immediately preserved in anticoagulant tubes containing EDTA, and stored at -20°C until DNA extraction. Genomic DNA was isolated from whole-blood samples following the standard phenol-chloroform extraction procedure described by Sambrook and Russel [27]. The concentration and purity of DNA were measured by a NanoDrop ND-1000 Spectrophotometer (DeNovix DS-11), and then DNA was stored at -20°C.

Primer design and gene amplification

Primers were designed to amplify specific exons in GPAM, DGAT1, and SCD1 genes using OligoCalc, IDT oligo analyzer, and the National Center for Biotechnology Information (NCBI) primer blast online software (Table 1). The complete gene sequence of the GPAM gene from *Bos taurus* (accession number: AH014852.2) and DGAT1 gene from *Bubalus bubalis* (accession number: NC_059171.1) were downloaded from the NCBI website (https://www.ncbi.nlm.nih.gov/). A bovine reference genome assembly (EMBL: AY241932) was used for designing the primers required to amplify exon-4 in the SCD1 gene.

The PCR reactions of GPAM, DGAT1, and SCD1, genes were performed in a DNA Engine thermal cycler (Bio-Rad, California, USA). The PCR reaction mixture for each gene included 100 ng of genomic DNA, 5 µL of 5 X Phusion™ High-Fidelity buffer, 1.25 µL of 10 µM per primer, 0.5 µL of 10 mM dNTPs, and 0.25 µL of Phusion™ High-Fidelity DNA polymerase, and nuclease-free water was added to the PCR reaction to make a total reaction volume of 25 µL. The PCR temperature gradient method was applied to determine the optimal annealing temperature for each primer set. PCR conditions for the GPAM gene started with an initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 40 sec, annealing phase at 49°C for 27 sec, and extension at 72°C for 45 sec, and then a final extension at 72°C for 7 min. The PCR protocol for DGAT1 gene amplification was similar to that of GPAM gene, except for annealing and extension conditions that were 56.2°C for 27 sec and 72°C for 35 sec, respectively. The cycling profile for the SCD1 gene involved an initial denaturation step at 98°C for 30 sec, followed by 30 cycles (denaturation at 98°C for 10 sec, primer annealing at 55°C for 25 sec, and an extension at 72°C for 30 sec), and a final extension at 72°C for 7 min.

The efficiency and specificity of PCR amplification were evaluated by agarose gel electrophoresis of 4 µL of PCR products. The amplified DNA fragments run on a 1.5% (w/v) agarose gel (Bio-Rad, California, USA) in 1X TAE buffer. A 100-bp ladder was used as a molecular weight marker. The gels were stained with ethidium bromide (0.5 µg/ml). The gel system was then run at 95 V for approximately 55 min using a Gel Documentation system manufactured by Syngene, the amplicons were viewed under ultraviolet light, and captured.

Single-strand conformation polymorphism analysis and sequencing

To perform SSCP analysis, the PCR products were separated, with optimization of various factors for each gene, including the quantity of PCR product, acrylamide concentration, denaturing solution composition, percentage cross-linking, voltage, temperature, glycerol, and running time. The denaturing solution, consisted of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol, and 10 mM NaOH, was mixed with each PCR product in a 1:1 ratio. The mixture was denatured at 95° C for 5 min, followed by a rapid cooling on ice. Both SCD1 and GPAM gene amplicons were loaded onto non-denaturing 13% polyacrylamide gels (29:1) and subjected to electrophoresis for about 16 h at 150 V in 1X Tris-borate-EDTA (TBE) buffer at 4° C. For the DGAT1 gene, a 10% acrylamide gel (29:1) was utilized and electrophoresis was conducted for 7 h at 180 V to improve amplicon resolution. Electrophoresis was performed for all genes using 1XTBE buffer in a vertical electrophoresis apparatus (AE-6530, ATTO Corporation, Korea). After running PCR-SSCP products, the gels were stained with silver nitrate [27], and dried on cellophane using the Bio-Rad Model 583 Gel Dryer (Bio-Rad, California, USA). Subsequently, the electrophoresis outputs were screened by a gel documentation system (Syngene, England), captured, and rated. If the target fragments of each studied gene were present in the PCR-SSCP electrophoresis bands, then the samples were processed for sequencing using the ABI PRISM 3730XL analyzer (Macrogen, Seoul, South Korea). The obtained sequences were analyzed by alignment using several free online software, including EMBL transseq (http://www.ebi.ac.uk/Tools/st/) and Jalview software version 2.11.3.2.

Data analysis

Various genetic parameters were calculated using GenAlEx Version 6.5 [28], including allele frequencies, effective number of alleles (Ne), observed heterozygosity (H_O), expected heterozygosity (H_e), Shannon's information index (I), fixation index (F), and assessment of Hardy–Weinberg equilibrium. Additionally, polymorphic information content (PIC) for each gene in each breed was calculated using Online Marker Efficiency Calculator (https://irscope.shinyapps.io/iMEC/) [29]. To estimate allelic richness (Rs), the hierfstat package in R was employed, taking into account variations in sample size among the buffalo populations under study. Given the large disparity in sample sizes, each population's genotypes were randomly subsampled to a standardized size of 35 individuals per group based on the method proposed by Foulley and Ollivier [30]. Furthermore, F_{ST} estimators, which are less sensitive to sample size variations, were calculated to examine the genetic differences among buffalo breeds. In this regard, Wright's F statistics, including the population differentiation coefficient (F_{ST}), total inbreeding coefficient (F_{IT}), and inbreeding coefficient (F_{IS}) were assessed using the hierfstat package in R, following the recommendations of Weir and Cockerham [31]. Also, pairwise F_{ST} analysis was conducted as a measure of genetic distance among populations.

Results and Discussion

Advances achieved in molecular biology in the last decades enabled the study of genetic variation at the DNA level and the identification of genomic regions underlying phenotypic variation in economically important traits in livestock. Polymorphisms of fat-related genes in buffalo have been reported in several breeds, including Murrah, Bhadawari, Toda, Pandharpuri, and Surti [32], Marathwada, Tarai, and Mehsana [33], and Italian Mediterranean buffalo [34]. In the present study, three key candidate genes of lipid metabolism (GPAM, DGAT1, and SCD1) in Murrah, Bhadawari, and Egyptian buffalo breeds were investigated. To characterize the SNPs of the exons 15, 17, and 4 of the GPAM, DGAT1, and SCD1 genes, respectively, The size of the amplified regions corresponding to GPAM, DGAT1, and SCD1 genes produced bands of 343 bp, 184 bp, and 312 bp, respectively. For GPAM, the PCR product size is composed of 265 bp for exon 15, in addition to 29 bp from the downstream and 49 bp from the upstream regions (https://www.ncbi.nlm.nih.gov/nuccore/AH014852.2) For SCD1, the PCR amplified product is comprised from 206 bp of exon 4, in addition to 61 bp from the preceding intron and 45 bp from the following intron (https://www.ncbi.nlm.nih.gov/nuccore/AY241932). The clear and specific bands of the expected sizes without non-specific bands were obtained and they indicate a successful amplification of the targeted exonic regions, demonstrating the specificity of the

primers and consistency in DNA extraction and amplification processes

Single-strand conformational polymorphism analysis

This study investigated nucleotide sequence variations in PCR products resulting from single-base substitutions using SSCP technique to explore the genetic diversity in GPAM, DGAT1, and SCD1 genes between Egyptian and Indian buffalo breeds. GPAM gene displayed two distinct SSCP patterns (Fig.1a): AA (expressed by two bands) and GA (includes three bands). In contrast, DGAT1 gene showed three patterns regarding both DD and GG genotypes (represented by two bands), while DG consisted of three bands (Fig.1b). Three different SSCP patterns were observed for the SCD1 gene (Fig.1c); AA and BB which are expressed by three bands, and AB that included four bands.

Allele and genotype frequencies

Table 2 shows both genotypic and allelic frequencies for each analyzed gene in buffalo breeds based on the genotyping data for GPAM, DGAT1, and SCD1 genes. The relative allele and genotype frequencies were estimated for the three genes under investigation. Specifically for GPAM gene, the highest frequency recorded for allele A occurs in Murrah buffalo, whereas allele G is present at a higher frequency in Egyptian buffalo compared to the two Indian breeds. Yu et al. [35], found a higher frequency of allele G in the Chinese Holstein dairy cattle. For the DGAT1 gene, Murrah breed exhibited the highest frequency of allele D, whereas Bhadawari breed displayed a higher frequency of allele G. The allele frequency recorded by Naserkheil *et al.* [36] in Iranian buffalo for allele G was lower than that obtained for the same gene in our study. On the other hand, the three buffalo breeds demonstrate a higher frequency at allele A than allele B for SCD1 gene. Previous studies on the SCD1 gene reported a higher frequency of allele A in Italian Mediterranean buffalo [37], Italian Holstein [38], Jersey, Valdostana cattle [39], Japanese Black cattle breed [40], and Canadian Holstein and Jersey cows [41]. On contrary, the frequency recorded of allele A in Fleckvieh bulls [42] and Italian Brown cattle [43] was lower than that obtained in our study.

Hardy-Weinberg Equilibrium (HWE)

The Hardy-Weinberg equilibrium was tested using the chi-square (χ^2) test. Tests for deviations from HWE for all breeds are illustrated in Table 3. A highly significant ($P < 0.001$) divergence from HWE was observed for GPAM, DGAT1, and SCD1 genes in the Egyptian buffalo breed. Furthermore, Bhadawari breed differed significantly ($P < 0.05$) from HWE in DGAT1 gene, whereas Murrah breed demonstrated a considerable deviation from HWE in SCD1 and DGAT1 genes. The analysis showed that HWE was not maintained in the studied populations. The tested individuals of Egyptian buffalo represented a chosen set of buffalo cows classified into high- and low-milk-fat content. Therefore, the observed deviation from HWE is justified. However, the effect of inbreeding is unlikely to be crucial as the animals were selected from different farms and unrelated sire families. On the other side, animals belonging to Murrah and Bhadawari buffalo breeds were sampled from a group of small farms that are geographically close to each other. So, the little deviation from HWE demonstrated by both Murrah and Bhadawari buffalo breeds may be due to population admixture and inbreeding [44]. A past study revealed that Murrah and Mediterranean buffalo did not differ significantly from HWE in DGAT1 gene, which contradicts our study findings [45]. Regarding SCD1 gene, Nili-Ravi buffalo presented significant differences from HWE at two polymorphic sites (p.2648, p.6596). These results are consistent with those obtained in the current study on Egyptian and Murrah breeds, but in discordance with Bhadawari breed [46]. Other studies revealed significant deviations from HWE ($P < 0.05$) at multiple microsatellite loci in Indian Nagpuri Buffalo [47] and Turkish water Buffalo [48].

Genetic Diversity

An overview of the statistical analysis of genetic diversity parameters is displayed in Table 4. Rs is a valuable parameter for assessing genetic diversity and conservation. It is defined as the total number of alleles observed in each population and the predicted allelic number to be absent within a given population [30]. In the current study, the values of Rs for GPAM, DGAT1, and SCD1 genes were 2, 3, and 3, respectively. This suggests uniformity in Rs among the studied breeds for these genetic loci, indicating a similar level of genetic diversity across the populations. Building upon the consistent Rs observed across populations for the relevant genes, we further analyzed observed (H_o) and expected (H_e) heterozygosity to assess genetic diversity. The H_o values for GPAM and DGAT1 genes ranged from 0.27 (Murrah) to 0.44 (Egyptian) and 0.23 (Egyptian) to 0.28 (Murrah), respectively, indicating considerable variations in genetic diversity among populations. Similarly, SCD1 gene displayed a wide range of H_o from 0.26 (Egyptian) to 0.45 (Bhadawari). In contrast, the H_e values for GPAM and DGAT1 genes showed the highest levels in the Egyptian buffalo (0.35 and 0.49, respectively), while for SCD1 gene it was consistent at 0.48 for Egyptian and Bhadawari breeds. Generally speaking, the estimates of both H_o and H_e for the three breeds remained below 0.5, implying a reasonable degree of genetic diversity within the investigated populations

and the presence of several polymorphic loci, as explained by Bhuyan et al. [49].

Expanding our exploration of genetic diversity, we utilized the Shannon information index (I) as a valuable indicator of heterozygosity within each population. For GPAM gene, the I value ranged between 0.39 (Murrah) and 0.53 (Egyptian), while for DGAT1 gene, similar I values of 0.65, 0.66, and 0.68 were recorded for Murrah, Bhadawari, and Egyptian buffalo breed, respectively. Shannon's index ranges between 0 and 1, where low values suggest that the breeds are more diverse, while high values point out to less diversity [50].

Continuing our analysis, we also considered the Polymorphic Information Content (PIC) parameter, which indicates a marker's level of informativeness. PIC values ranged from 0.2 to 0.3 for GPAM, while for DGAT1, Bhadawari had the highest PIC value (0.48) recorded in the present study. Moderate and similar PIC vales were obtained for the Egyptian (0.38) and Murrah (0.37) breeds. Conversely, for SCD1 gene, PIC values fell within the higher range (0.3 and 0.4). A high PIC value indicates that a locus with numerous alleles evenly distributed in frequency, thus enhancing the locus informativeness. In population genetic studies, genetic markers with PIC values less than 0.25 are considered less informative, whereas those exceeding 0.5 are deemed highly informative [51].

Furthermore, we examined the Fixation Index (F_{IS}), which quantifies the extent of inbreeding within a group. Across all studied populations, positive F_{IS} values were calculated for both DGAT1 and SCD1 genes, indicating a tendency toward inbreeding. In contrast, negative F_{IS} values were recorded for GPAM gene, implying limited inbreeding rates [48, 52]. Positive (0.78) and negative (-0.15) F_{IS} value for GPAM gene were recorded in Murrah buffalo and Mediterranean buffalo, respectively [45].

Population Differentiation

Values of F statistics (F_{ST}, F_{IT}, and F_{IS}) for the total populations are demonstrated in Table 5, providing further insights into genetic diversity and population structure. F_{ST} quantifies the degree of genetic differentiation among the studied populations, with values ranging from -0.01 (SCD1) to 0.05 (DGAT1). Basically, F_{ST} measures the fraction of genetic variance attributable to population variation, where 1 denotes complete genetic distinctiveness and 0 implies genetic similarity among populations [53]. F_{IT} estimates genetic variation within and across populations, reflecting the total amount of inbreeding across the entire sample. In the current study, F_{IT} values ranged between -0.22 (GPAM) and 0.50 (DGAT1), providing insights into the overall genetic diversity and inbreeding levels within the studied populations [53]. Notably, positive F_{IS} values were observed for SCD1 and DGAT1 genes, highlighting an increased departure from HWE due to factors such as selection, population structure, and/or non-random mating [54]. F_{IS} measures the within-population inbreeding level, with positive values reflecting a larger departure from HWE. For DGAT1 gene, our results are in discordance with the F_{IS} coefficients belonging to the Ethiopian cattle [55], and Beninese Borgou cattle [56]. Regarding SCD1, our findings indicate a positive F_{IS} estimate, which is consistent with the positive F_{IS} value of the Italian Brown cattle [43] and Indonesian Holstein-Friesian cows [57], while opposite to the negative F_{IS} value calculated for some Italian cattle breeds [58]. The wide variation observed in F_{IS} values among studies may be due to selection and accumulated inbreeding.

Genetic Distance

The use of Weir and Cockerham method for F_{ST} calculation allowed for comparing population differentiation among the studied buffalo populations. Fig.2 shows the genetic distance, represented by F_{ST} values, in pairwise comparisons between the three breeds. Notably, the shortest genetic distance (0.002) was observed between Egyptian and Murrah breeds, while the longest distance (0.056) was calculated between Bhadawari and Murrah breeds. Genetic distance serves to measure genetic similarities and differences between species, breeds, strains, and populations, facilitating the confirmation of pedigree relationships, genetic characterization of breeds or strains, and assessment of species variation over time [59]. Our findings demonstrate a very short genetic distance between the Egyptian buffalo and Indian Murrah buffalo, implying their close genetic relationship. This supports similarities in the origins of the two genetic groups, possibly resulting from immigration, mutation, and/or organized crossbreeding. While these insights offer valuable contributions to our understanding of the genetic dynamics and evolutionary history of river buffalo populations, it is important to acknowledge the limitations posed by our study. The small sample size for each studied breed along with the limited genome coverage by the relevant candidate genes analyzed restrict the extent to which definitive and solid conclusions can be drawn. Further research with larger sample sizes and comprehensive genome coverage is necessary to provide a more robust understanding of the genetic dynamics and evolutionary patterns within the river buffalo populations.

Mutation detection and analysis of sequences in the Egyptian buffalo breed

Nucleotide sequences were translated into the corresponding peptide sequences using the EMBOSS Transeq program, to ascertain whether the SNPs discovered were synonymous or non-synonymous as shown in Table 6. After comparing the sequenced fragments of GPAM gene with the reference sequence of *Bos taurus* available at NCBI under accession number AH014852.2 (Fig.3a), four observed SNPs were identified in GPAM gene in Egyptian buffalo samples (SNP1-g.150T>C, SNP2-g.182G>C, SNP3-g.297A>C, and SNP4-g.318A>T), and one of them lead to non-synonymous (missense) amino acid changes. As shown in Table 6, SNP at position G182C caused arginine (R) to be substituted with proline (P), and the other one at position A318T caused a change in amino acid from leucine (L) to phenylalanine (F). Studies on the polymorphisms of GPAM gene have received much less attention than studies on the gene's function. A past study analyzed GPAM gene in Murrah, Bhadawari, Pandharpuri, Toda, and Surti Indian buffalo breeds, and reported amino acid substitutions in most breeds [19]. Similar SNPs in GPAM have been associated with characteristics related to fat content and milk yield in the Chinese Holstein cattle [35], Chinese Simmental cattle [60], and Korean native cattle [61].

Regarding DGAT1 gene, the sequenced amplicons were aligned with the reference sequence assembly of the water buffalo (NC_059171.1) available at NCBI. Two SNPs (SNP1-g.106 C>T, and SNP2-g.123C>T) were identified (Fig.3b), resulting in non-synonymous substitutions, such as R to cysteine (C) at position C106T (Table 6). Previous studies have linked particular SNPs at DGAT1 gene to milk production traits in Murrah, Tarai, Bhadawari, Pandharpuri, Mehsana, Marathwada, Surti, Jaffarabadi buffalo [33, 62] , Anatolian water buffalo [11], Iranian buffalo [63], Amazon buffalo breeds [45], and Chinese water buffalo [18].

The sequenced fragments of SCD1 gene were also compared with the bovine reference assembly (NCBI accession number: AH011561.2). Six SNP substitutions were detected, including SNP1-g.124C>T, SNP2-g. 127C>T, SNP3-g.201A>T, SNP4-g.214C>T, SNP5-g.237G>A, and SNP6-g.253G>A (Fig.3c). The changes identified in DNA sequences generated substitutions in amino acid sequences such as (SNP1) R to be substituted with serine (S), (SNP2) P deletion, (SNP3) glutamine (Q) to histidine (H), (SNP4) H to tyrosine (Y), and, (SNP6) aspartic (D) to asparagine (N), as shown in Table 6. Past studies associated variations detected in SCD1 gene are associated with milk traits, meat quality, and fatty acid composition in Mediterranean river buffalo [10], Khuzestan buffalo population [64], Italian Mediterranean buffalo [37], and Canadian Holstein and Jersey cows [41].

Conclusion

The present study revealed significant genetic diversity in GPAM, DGAT1, and SCD1 genes among Egyptian, Murrah, and Bhadawari buffalo breeds. Distinct PCR-SSCP patterns and validated SNPs indicate substantial polymorphisms with potential functional implications. Sequencing identified four SNPs at GPAM, two SNPs at DGAT1, and six SNPs at SCD1, resulting in non-synonymous amino acid substitutions in the Egyptian buffalo breed. The observed genetic variability and deviations from Hardy-Weinberg equilibrium reflect possible influences of selection, genetic drift, and/or non-random mating on population structure. Wright's F statistics and genetic distance analysis highlight a close relationship between the Egyptian and Murrah buffalo breeds. These findings support the importance of genetic diversity studies for establishing effective conservation schemes and sustainable breeding programs in buffalo populations. The identified genetic variants in GPAM, DGAT1, and SCD1 genes offer valuable insights to enhance buffalo milk quality through targeted breeding programs.

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Ethical approval

This study was approved by the Institutional Animal Care and Use Committee (CU-IACUC) of Cairo University, Egypt (approval number: CU-II-F-45-22).

TABLE 1. Primer pairs for amplification of GPAM, DGAT1, and SCD1 in buffalo breeds

Gene	Exon	Primer sequences (5' - 3')	PCR product size (bp)
GPAM	15	**F:** CATGAGACCACTTCTGTC	343
		R: GCTTCTGGCCACTTGATT	
DGAT1	17	**F:** GATAGTGGGCCGCTTCTT	184
		R: CAAAGCAGTCCAACACCCA	
SCD1	4	**F:** TCTGGAAGACACCTGGCAA	312
		R: CCAGCTCCATCATCATGTC	

GPAM: Glycerol-3-Phosphate Acyltransferase, Mitochondrial, DGAT1: Diacylglycerol O-Acyltransferase 1, SCD1: Stearoyl-CoA Desaturase 1, F: forward, R: reverse, bp: base pairs.

TABLE 2. Genotype and allele frequencies of the detected polymorphisms on GPAM, DGAT1, and SCD1 genes in buffalo breeds

Gene	Breed	No.	Genotype frequency			Allele frequency	
			AA	**GA**	**GG**	**A**	**G**
GPAM	**Egyptian**	140	0.56	0.44	-	0.78	0.22
	Murrah	60	0.73	0.27	-	0.87	0.13
	Bhadawari	40	0.70	0.30	-	0.85	0.15
			DD	**DG**	**GG**	**D**	**G**
DGAT1	**Egyptian**	140	0.47	0.23	0.30	0.59	0.41
	Murrah	60	0.50	0.28	0.22	0.64	0.36
	Bhadawari	40	0.22	0.28	0.50	0.36	0.64
			AA	**AB**	**BB**	**A**	**B**
SCD1	**Egyptian**	140	0.47	0.26	0.27	0.60	0.40
	Murrah	60	0.47	0.33	0.20	0.63	0.37
	Bhadawari	40	0.38	0.44	0.18	0.60	0.40

GPAM: Glycerol-3-Phosphate Acyltransferase, Mitochondrial, DGAT1: Diacylglycerol O-Acyltransferase 1, SCD1: Stearoyl-CoA Desaturase 1.

TABLE 3. P-values for Pearson's χ^2 test of Hardy Weinberg equilibrium

Gene	Breed	ChiSq	P-value
GPAM	**Egyptian**	11.324	0.001***
	Murrah	1.420	0.233 ^{NS}
	Bhadawari	1.246	0.264 ^{NS}
DGAT1	**Egyptian**	39.180	0.000***
	Murrah	8.841	0.003**
	Bhadawari	6.561	0.010*
SCD1	**Egyptian**	30.179	0.000***
	Murrah	4.781	0.029*
	Bhadawari	0.156	0.693 ^{NS}

GPAM: Glycerol-3-Phosphate Acyltransferase, Mitochondrial, DGAT1: Diacylglycerol O-Acyltransferase 1, SCD1: Stearoyl-CoA Desaturase 1, NS: non-significant, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

TABLE 4. Genetic diversity of the studied buffalo breeds

Gene	Breed	No.	Ne	Rs	Ho	He	I	PIC	F_{IS}
GPAM	**Egyptian**	140	1.53	2	0.44	0.35	0.53	0.29	-0.28
	Murrah	60	1.30	2	0.27	0.23	0.39	0.20	-0.15
	Bhadawari	40	1.34	2	0.30	0.26	0.42	0.22	-0.18
DGAT1	**Egyptian**	140	1.94	3	0.23	0.49	0.68	0.38	0.53
	Murrah	60	1.85	3	0.28	0.46	0.65	0.37	0.38
	Bhadawari	40	1.86	3	0.28	0.46	0.66	0.48	0.41
SCD1	**Egyptian**	140	1.92	3	0.26	0.48	0.67	0.39	0.46
	Murrah	60	1.87	3	0.33	0.46	0.66	0.38	0.28
	Bhadawari	40	1.92	3	0.45	0.48	0.67	0.41	0.06

GPAM: Glycerol-3-Phosphate Acyltransferase, Mitochondrial, DGAT1: Diacylglycerol O-Acyltransferase 1, SCD1: Stearoyl-CoA Desaturase 1, No.: number of genotyped animals, Ne: effective number of alleles, Rs: allelic richness, Ho: observed heterozygosity, He: expected heterozygosity, I: Shannon's Information Index, PIC: polymorphic information content, and F_{IS}: fixation index.

TABLE 5. Estimates of F statistics across the three buffalo breeds at each locus

Gene	F_{ST}	F_{IT}	F_{IS}
GPAM	0.01	-0.22	-0.24
DGAT1	0.05	0.50	0.48
SCD1	-0.01	0.35	0.36

GPAM: Glycerol-3-Phosphate Acyltransferase, Mitochondrial, DGAT1: Diacylglycerol O-Acyltransferase 1, SCD1: Stearoyl-CoA Desaturase 1, F_{ST}: population differentiation coefficient, F_{IT}: total inbreeding coefficient, F_{IS}: inbreeding coefficient.

TABLE 6. Characteristics of single nucleotide polymorphism diversity in selected gene fragments detected by SSCP and sequencing

Gene	PCR product size (bp)	SNP order	Nucleotide change	Amino acid change
GPAM	343	150	T > C Substitution	No change
		182	G > C Substitution	Arginine / Proline
		297	A > C Substitution	No change (Intron)
		318	A > T Substitution	No change (Intron)
DGAT1	184	106	C > T Substitution	Arginine / Cysteine
		123	C > T Substitution	No change
SCD1	312	124	C > T Substitution	Arginine / Serine
		127	C > T Substitution	Deletion (Proline)
		201	A > T Substitution	Glutamine / Histidine
		214	C > T Substitution	Histidine / Tyrosine
		237	G > A Substitution	No change
		253	G > A Substitution	Aspartic / Asparagine

GPAM: Glycerol-3-Phosphate Acyltransferase, Mitochondrial, DGAT1: Diacylglycerol O-Acyltransferase 1, SCD1: Stearoyl-CoA Desaturase 1, SNP order: indicates the SNP order relative to the starting point of the PCR product, bp: base pairs.

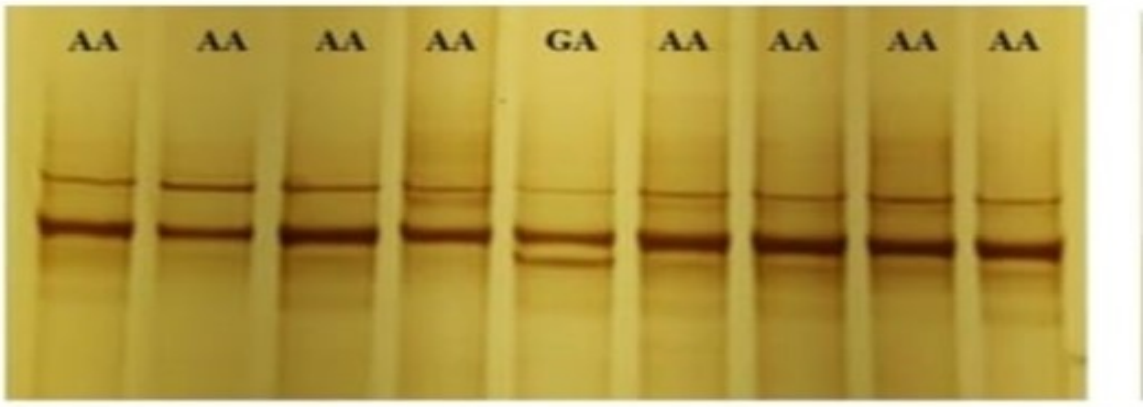


Fig.1a PCR-SSCP patterns of GPAM gene.

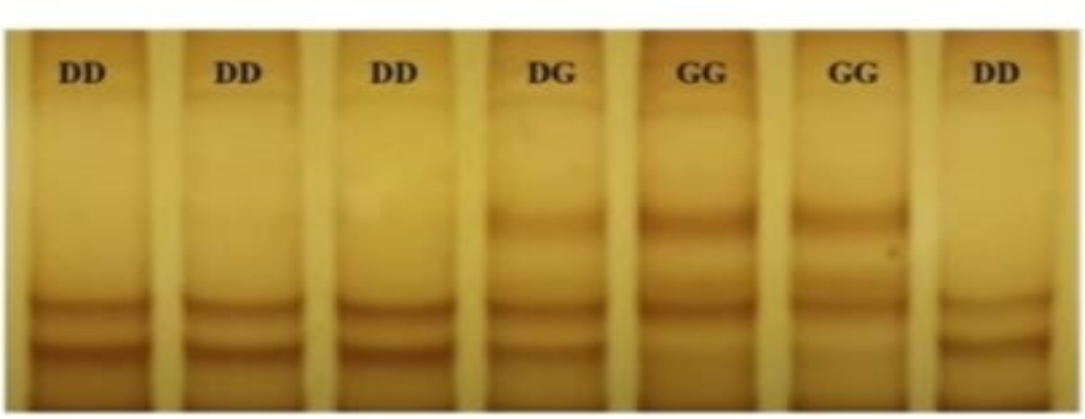


Fig.1b PCR-SSCP patterns of DGAT1 gene.

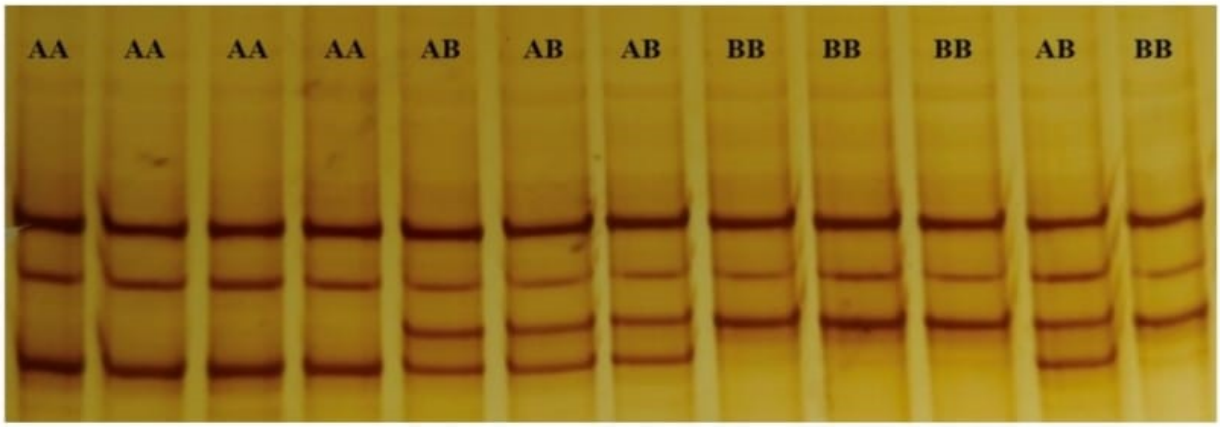


Fig.1c PCR-SSCP patterns of SCD1 gene.

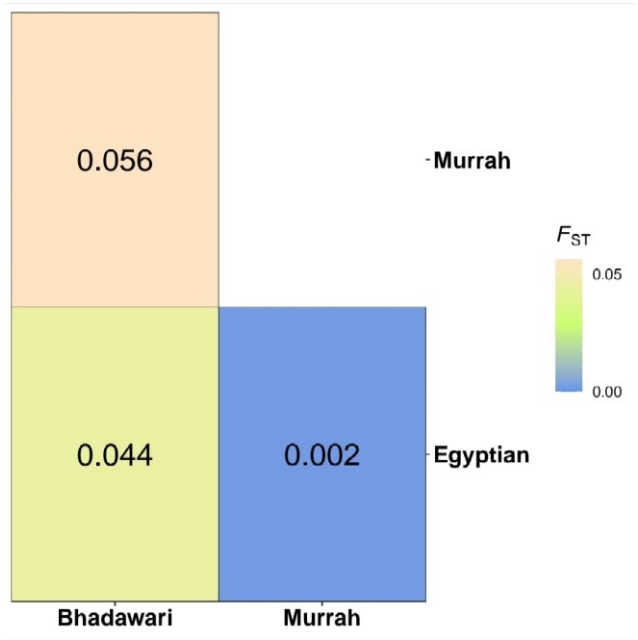
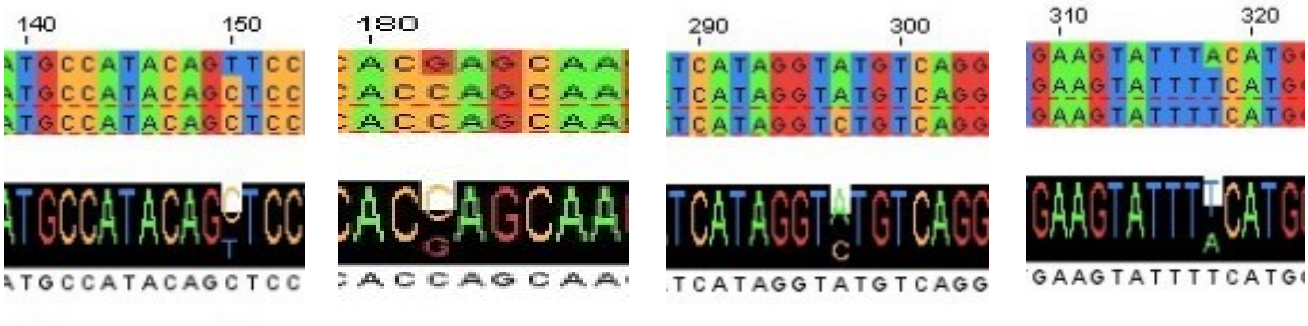
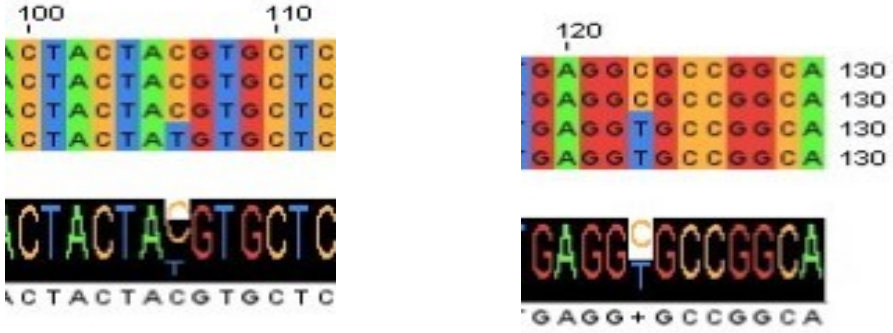


Fig.2 Pairwise F_{ST} distances among the three buffalo breeds under investigation.



SNP1-g.150T >C **SNP2-g.182G>C** **SNP3-g.297A** **SNP4-g.318A>T**

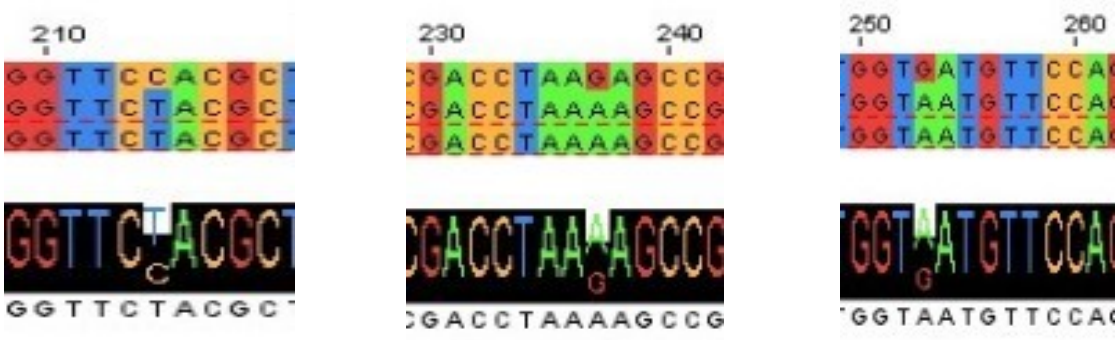
Fig.3a SNPs in exon 14 of GPAM gene.



SNP1-g.106 C>T **SNP2-g.123C>T**

Fig.3b. SNPs in exon 17 of DGAT1 gene.

SNP1-g.124C>T **SNP2-g. 127C>T** **SNP3-g.201A>T**



SNP4-g.214C>T **SNP5-g.237G>A** **SNP6-g.253G>A)**

Fig.3c SNPs in exon 4 of SCD1 gene

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التنوع الوراثي فى الجينات المرشحة لتكوين دهن اللبن GPAM و DGAT1 و SCD1 في جاموس النهر

سونيا سيهاج[1] ، محمد عادل زهران[2*] ، حمدي عبد الشافي[2] ، فينود شوكار [1] ، أنيل كومار [1] ، سامي أبو بكر[2]، محمد صابر حسنين [3] و حسام الدين رشدى [2]

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الملخص

تهدف هذه الدراسة إلى الكشف عن تعدد الأشكال المظهرية لجينات الميتوكوندريا جليسيرول -3- فوسفات أسيل ترانسفيراز (GPAM)، داي أسيل جليسيرول -أُو- أسيل ترانسفيراز **1** (DGAT1)، بالإضافة لجين سترويل كو انزيم ايه ديساتيوريز 1 (SCD1) في الجاموس المصري، وكذلك دراسة التنوع الوراثي بين سلالات الجاموس المصري Egyptian buffalo ، وجاموس مورا Murrah، وجاموس بهداواري Bhadawari. تم إجراء التنميط الجيني باستخدام تقنية PCR-SSCP وطريقة التسلسل الجينى gene sequencing. تشيرالنتائج إلى انحرافات كبيرة عن توازن هاردي- فاينبرج في الجاموس المصري فى الجينات الثلاثة محل الدراسة، مع وجود انحراف فى عشيرة جاموس مورا بشكل كبير في جينات SCD1، DGAT1. بالإضافة لذلك، وجد أن جين DGAT1 هو الجين الوحيد الذي ينحرف بشكل كبير عن اتزان هاردى فاينبرج في سلالة بهداواري. كذلك كشفت المقارنة الزوجية بين السلالات الثلاثة عن مسافات جينية متفاوتة، مع ملاحظة أن أقصر مسافة وجدت كانت بين الجاموس المصري وجاموس مورا ، فى حين أن أطول مسافة كانت بين سلالة بهداواري ومورا. كذلك تم تحديد تعدد الأنماط الوراثية الناتج عن التغير على مستوى النيوكليوتيدة الواحدة (SNP) في الجينات الثلاثة فى الجاموس المصرى، مع تحديد مايترتب على ذلك من تغير فى الأحماض الأمينية. تكشف هذه النتائج عن اختلافات جينية جديدة ذات آثار محتملة على عملية تمثيل الدهن وجودة اللبن في الجاموس المصري. لذلك لابد من إجراء المزيد من الدراسات لفهم الأهمية الوظيفية لهذه الاختلافات وكيفية الإستفادة منها فى برامج التحسين الوراثى لإنتاج اللبن الجاموسى.

الكلمات الدالة: التنوع الوراثى، تعدد الأشكال المظهرية، التنميط الجيني، جاموس النهر، دهن اللبن.