# ASSOCIATION OF NEW SNPs AT DGAT1 GENE WITH MILK QUALITY IN EGYPTIAN ZARAIBI GOAT BREED

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## SUMMARY

*This study aimed to detect putative genomic loci in candidate genes associated with milk composition in Egyptian Zaraibi goats. A total number of 50 samples were tested to detect polymorphism in exons 15 and 16 of the diacylglycerol acyltransferase 1 (DGAT1) gene. The PCR products of different electrophoresis patterns were sequenced and aligned with multiple sequence alignment tools (Clastal Omega and Jalview). Sequence analysis showed three new genotypes in the studied samples: the first was BB with C12T and G219A SNPs, the second DD contains C84T and G219A SNPs and the third was AG genotype with only G219A SNP. General Linear Model Analysis (GLM) showed that the DD genotype group with C84T and G219A SNPs had significantly the highest fat percent. Meanwhile, BB genotype group with C84T and G219A SNPs recorded significantly the highest total solids levels. On the other hand, the AG genotype group which has G219A SNP showed non-significant effect on milk components. Those new SNPs were submitted to GenBank and approved by accession number OM418856, OM418857 and OM418858 to be published. Moreover, translation of those sequences showed that the G219A SNP causes a substitution of Glycine to Serine in exon 16 at position 106. This SNP (G106S) was predicted to be tolerated by SIFT with a score of 0.48. Substitution of Glycine (which is a small amino acid located on the surface of the protein) to Serine (which is larger than glycine) could lead to losses of several interactions with adjacent molecules in the protein structure.*

*In conclusion, the results demonstrated several genetic variations in DGAT1, which are associated with different milk composition, so it could be used as genetic markers for the selection of dairy goats in breeding programs to improve milk quality.*

***Keywords: DGAT1 gene, polymorphism, milk composition, Zaraibi goats***

## INTRODUCTION

Goats are considered as one of the most efficient ruminant species that adapt to both tropical and desert environments (Metawi 2011; Kaliber *et al*., 2016). In addition, they are less expensive and easier to keep than other livestock in developing countries, where space and capital are limited (Khalil *et al*., 2013). Moreover, they present a significant economic interest in many countries (Selvaggi *et al*., 2014; Chen *et al*., 2019), where its milk is desirable for many people around the world due to its richness in various nutrients, as well as, it is relatively often used as an alternative to cow milk because of its ease in digestibility and low allergenicity (Clark and Mora Garcia, 2017). Milk production traits have fundamental importance in livestock production and the related economy (Erhardt*et al*., 2010 and An *et al*., 2012). Milk fatty acids play an essential role in cheese making and its quality, it has been found that milk fat includes about 98% triglycerides (TGs) (Mansson, 2008). The final and the only committed step in the biosynthesis of Triglycerides (TGs) are catalyzed by Diacylglycerol acyltransferase (DGAT) enzymes (DGAT1 and DGAT2) as reported byCases *et al*. (2001). Acyl CoA: diacylglycerol acyltransferase (DGAT1) gene was reported as a production-associated gene in several animals including buffalo, sheep and goat (Mansson, 2008). DGAT1 gene is expressed in nearly all tissues, including the mammary glands (Khan *et al.,* 2021) and acts as a catalyst to triacylglycerol synthesis, which has an essential role in milk fat metabolism (Mohamed, 2016). Increasing productive performance through genetic selection is a common goal for many animal breeding programs worldwide (Meredith *et al*., 2012, Narayana *et al*., 2017 and Heimes *et al*., 2019). In order to improve productivity, animals with better quality traits such as milk production, growth, meat, and carcass quality have been selected and used in the breeding program in the animal industry. Selection aimed to increasing the frequency of alleles with a positive effect on a given trait initiated by geneticists (Dekkers, 2004).

In goats, the DGAT1 gene (Gene ID: 100861225) is located on chromosome 14 with 18 exons (Khan *et al.*, 2021). Several studies reported single nucleotide polymorphisms (SNPs) in the goat DGAT1 gene; An *et al*. (2012) detected different points of mutation were (ins. C) at 407-408 in intron 14, C6852T and

C6798T at exon 7, where those SNPs showed a significant effect on fat percentage and milk yield. Consistently, Martin *et al*. (2017) observed two SNPs R251L and R396W in DGAT1 in Saanen and Alpine goat. They reported that the two types of mutation lead to substitution in the protein sequence and were associated with a decrease in milk fat but this association was not strongly significant. Moreover, four polymorphisms (T21153G, C21154G, A21172C, and A21194T) were identified in the Iranian Khalkhali goat. The variants T21153G and C21154G caused the transformation of serine to glycine amino acids, while A21172C was associated with the change in aspartic acid to alanine amino acid (Evrigh *et al.,* 2018). Oppositely, Ozmen and Kul (2014) detected T to C mutation in intron 16 of goat DGAT1 locus with no significant effect for TT and TC genotypes on milk yield, fat and lactose. In addition, Tabaran *et al*. (2014) reported no significant differences in milk fat percent among three genotypes CC, TT, and CT genotypes which were observed in the exon 17 of DGAT1 genes of the Turcana goat breed.

In Egypt, Zaraibi goats are considered the most famous native breed due to their productive and reproductive capabilities. It produces milk fat content ranged 2.6- 3.9% during 210 days lactation periods (Teleb *et al*., 2016). Identifying and validating genetic markers for milk production traits in Zaraibi goats are the initial and crucial steps to establish a marker assisted selection system (MAS). Eid*et al*. (2020) detected two important mutations (T703C and T713C) at DGAT1 in Zaraibi goats. They found that polymorphism T713C at the coding region was associated with the substitution of amino acid isoleucine to threonine, which significantly regulated the milk total solid and milk yield. This work is a complementary to previous study on DGAT1 gene polymorphism in Zaraibi goats (Eid et al., 2020). Using different samples, this study aims to investigate new SNPs in DGAT1 gene and their association with milk quality.

## MATERIALS AND METHODS

### *Animals 'management and ethical considerations:*

Fifty Zaraibi does reared in Sakha experimental station - Kafr El-Sheikh governorate - Animal Production Research Institute (APRI) - Agriculture Research Center (ARC) were used in this experiment. Does weight range from 28 to 42 kg and aged 24 to 108 months. All animals were fed according to NRC (2007) allowances. Water and mineral blocks were available all the times. Handling and protection of animals were done according to the recommendations of European Union directive 86/609/EEC (Louhimies, 2002).

### *Milk and blood samples:*

Milk samples were collected from all does during morning milking at days 90, 120, and 210 after kidding. Moreover, ten ml of blood samples were collected from does on ethylene diamine tetra acetic acid (EDTA) as an anticoagulant and kept at -20°C for DNA extraction.

### *Chemical analysis:*

The percentage of milk components (fat, protein, lactose, total solids, and solids not fat) were estimated in fresh samples using infra-red spectroscopy (Milko-Scan 133B; N. Foss Electric, DK 3400 Hillerod, Denmark) according to Smith *et al.,* 1993 and Lynch *et al.,* 2006.

### *DNA Extraction and PCR Amplification:*

All chemicals used were with molecular biology gradient and were purchased from Sigma Scientific Services co. (Cairo, Egypt).

Genomic DNA was extracted from all collected blood samples using salting-out method as described by Miller *et al*., 1988. The concentrations and purity of extracted DNA were measured using spectrophotometer (Eppendorf Biophotometer plus).
PCR Amplification was performed using Bio-Rad thermal cycler (model C1000). According to DGAT1 goat sequence (accession no.DQ380250.1), pairs of primers F, 5′CCCAGACACTTCTACAAGCC3′ and R 5′ TGCCCGATGATGAGTGACAG3′, were designed to amplify the region between exon 15 to 17 and the entire intron. PCR reaction was carried out in a 50 µl reaction mixture containing 150 ng genomic DNA, 25 µl of Dream TaqPCR Master Mix2x (Cat. No.: BIO-25043) and 10 pmol of each primer.PCR conditions were as follows: denaturation at 94 °C for 4 min; followed by 34 cycles of denaturation at 94 °C for 1 min; annealing at 53.5 °C for 1 min; extension at 72 °C for 1 min and final extension step at 72 °C for 10 min. PCR amplicons were electrophoresed in 1% agarose gels, using 1X TBE buffer containing 200 ng/ml of ethidium bromide, then visualized under UV light and photographed by digital camera.

### *Sequence analysis and SNP detection:*

PCR products were purified and sequenced by automated DNA ABI Prism 3130 Genetic Analyzer (Sanger *et al.,* 1977). SNPs were detected by comparing fragment sequences against the corresponding DGAT1 gene for goat (*acc. no. DQ380250.1*) on GenBank and analyzed using Cluster omega, Jalview 2.11.1.6 and BioEdit 7.0.5.3 sequence alignment editor programs (Hall, 1999). Analysis of the deleterious effect of non-synonymous SNP (nsSNP) was performed with SIFT, its scores was classified as damaging (0–0.1), borderline (0.101–0.2), or tolerant (0.201–1.00) http://sift.jcvi.org/, Choi and Chan, 2015).

### *Statistical analyses:*

Analysis of variance and least-squares means were obtained using the General Linear Model (GLM) procedure of SAS (2004). The effects of milking time, parity and DGAT1 genotypes on milk composition were assessed using the following linear model:

$Y_{iikl} = \mu+T_i+P_i+GD_k+e_{iikl}$

where$\mu$ = the overall mean; Y = the observed records on milk composition; $T_i$ = the fixed effect of $i^{th}$ time

of milk composition (i = 90, 120, 210); $P_j$= the fixed effect of jth parity of does (j = 1,…7); $GD_k$ = the fixed effect of $k^{th}$ DGAT1 genotype (k = AG, BB, DD); and $e_{ijkl}$ = the random error. The least squares mean of the genotypes were compared by the Tukey-Kramer test.

## RESULTS AND DISCUSSION

A total of 50 does blood samples were genotyped for polymorphism in the region between exon 15 and 17 including entire introns of the DGAT1 gene. The PCR product successfully amplified the 390 bp fragment (Fig.1).

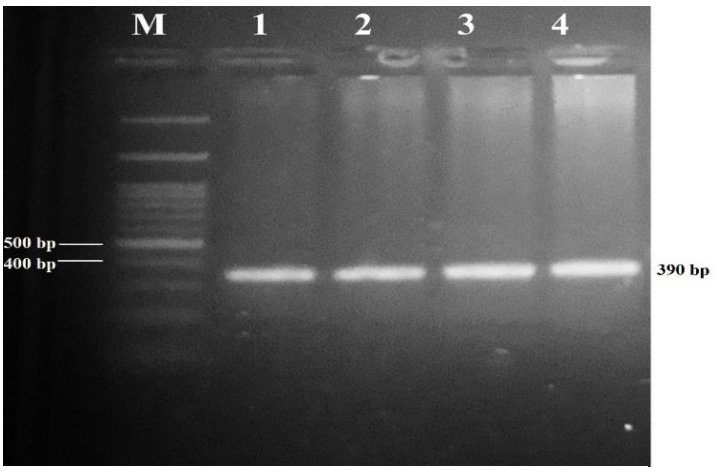


**Fig. 1. The electrophoric pattern of PCR amplification of DGAT1 gene, M: 100 bp DNA ladder, Lanes 1-4: 390 bp PCR product of DGAT1.**

***Sequence analysis and mutation detection:***

Sequence analysis showed three SNPs in the studied samples. The first one from cytosine (C) to thymine (T) at position 12 which give only one genotype C1T1, the second from C to T at position 84 which give C2T2 genotype only and the last mutation was from guanine (G) to adenine (A) at 219 gives one AG genotype. According to those SNPs, animals were divided into three groups; the first group was BB which contains C1T1 and AG genotypes (fig. 2a), the second DD contains C2T2 and AG genotypes (fig. 2b), and the third (AG) contains AG genotype only (fig. 2c). The new SNPs were submitted to GenBank and approved by accession number *OM418856, OM418857 and OM418858* to be published. Angiolillo *et al.* (2007) observed T 703 C SNP in intron 16 of DGAT1 gene of Spanish goats when sequencing region between exon 12 and 17. Moreover, Yang *et al.* (2011) recorded three genotypes TT, CC, and CT in four Chinese sheep breeds and a SNP (C→ T) in exon 17 of the DGAT1 gene, which is synonymous mutation as it didn't cause any substitution in amino acids. Similarly, Tabaran *et al.* (2014) observed three genotypes CC, TT, and CT in Turcana goat breed. Meanwhile, different polymorphisms were reported by Evrigh *et al.* (2018) at exon 17 were T110G, C111G, A129C, and A151T in Iranian Khalkhali goat. On the other hand, Eid*et al.* (2020) observed two mutations (T703C and T713C) at the same region of DGAT1 in Zaraibi goats.

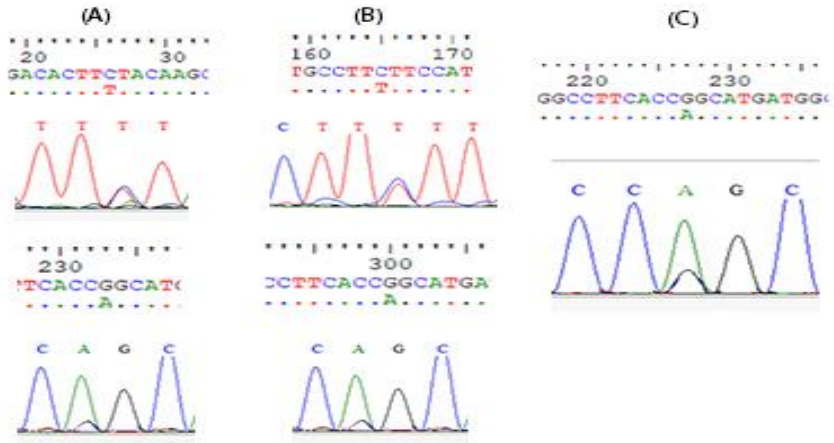


**Fig. 2. Genotyping of exon 16 region of DGAT1gene. (A) SNPs T12C and G219A in genotype BB (B) SNPs T84C and G219A in genotype DD. (C) SNP G219A in genotype AG.**

***Sequence translation and amino acid substitution:***

Sequences were translated to detect whether observed SNPs were synonymous or non-synonymous. Results showed that the GGC to AGC SNP at position 219 in studied sequence present in all samples causes a substitution of Glycine to Serine in exon 16 at position 106 in final protein, so it is considered as non-synonymous. This substitution (G106S) was predicted to be tolerated by SIFT with a score of 0.48. Meanwhile, the two mutations C12T and C84T didn't present in the coding region. Evrigh *et al.* (2018) found that transformation of amino acids from serine to glycine resulted from variants T110G and C111G, while A21172C was associated with the change in aspartic acid to alanine amino acid in Iranian Khalkhali goat. Moreover, Eid*et al*. (2020) reported that the polymorphism T713C at exon 16 at DGAT1 gene in Zaraibi goats was associated with the substitution of amino acid isoleucine to threonine. The results of the current study, showed that the substitution of Glycine (which is a small amino acid located on the surface of the protein) to Serine (which is larger than glycine) could lead to losses of several interactions with adjacent molecules in the protein structure.

***Effect of DGAT1 polymorphism on milk composition:***

Results illustrated in Table 1 showed that the DD group has significantly the highest fat percent (3.53±0.07%, $P<0.05$) that could be related to the effect of both C84T and A219G SNPs. Meanwhile, BB group recorded significantly the highest total solids levels (11.83±0.19%, $P<0.05$) suggesting that it is due to the presence of C84T and A219G SNPs. On the other hand, the AG group which has A219G SNP showed non-significant effect on milk components. In agreement, Eid*et al.* (2020) reported that the total solid content of milk is significantly regulated by SNP in exon 16. Oppositely, Ozmen and Kul (2014) observed non-significant effect of TT and TC genotypes on milk yield, fat and lactose levels in six goat breeds in Syria. Moreover, Tabaran *et al*. (2014) reported non-significant differences in milk fat percent in Turcana sheep and Carpatian goat breeds in Romania. In the current work, the two mutations C12T and C84T, which was not presented in the coding region, have a positive effect on milk production traits, suggesting that it might be due to its regulatory effect on the mechanism of mRNA.

**Table 1. LSM (±SE) of milk composition in Zaraibi does as affected by DGAT1 genotypes**

| Groups | Milk Composition (%) | | | | |
|---|---|---|---|---|---|
| | **Fat** | **Protein** | **Lactose** | **Total solid** | **Solid not-fat** |
| **BB** | 3.21±0.08[b] | 2.81±0.07 [a] | 4.09±0.07 [a] | 11.83±0.19 [a] | 7.60±0.09 [a] |
| **DD** | 3.53±0.07[a] | 2.80±0.06 [a] | 4.08±0.07 [a] | 10.04±0.18 [b] | 7.49±0.08 [a] |
| **AG** | 2.82±0.18[c] | 2.44±0.15 [a] | 3.95±0.16 [a] | 9.65±0. 42 [c] | 6.97±0.20 [a] |
| **Significance** | *S($P<0.001$) | NS | NS | *S($P<0.01$) | NS |

a, b and c= Means with the different letters in the same column are significantly different, *S= significantly different, NS=non significantly different.

## CONCLUSION

Three new SNPs (C12T, C84T, and A219G) within three genotypes C1T1, C2T2, and AG, respectively were recorded in this study. The highest milk total solids was associated with C1T1 and AG variants observed in group BB. Meanwhile, the highest value of milk fat was observed to be associated with C2T2 and AG variants recorded in group DD. Meanwhile, SNPs A219G was a non-synonymous which altered amino acid Gly. to Ser. (G106S), this amino acid altered the final protein structure. Considering fats and total solids, they are associated with milk quality which reflected the economic importance of the milk on the livestock industry. The new DGAT1 polymorphisms described here could be used as genetic marker for improvement of milk and cheese quality in goats.

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## إرتباط الاختلافات النيوكلتيدية في جين DGAT1 بجودة اللبن في سلالة الماعز الزرايبي المصرية

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تهدف هذه الدراسة إلى تحديد المواقع الجينومية في بعض الجينات المعروفة ذات الصلة بمكونات اللبن في الماعز الزرايبي المصرية .تم إختبار ٥٠ عينة لتحديد الاختلافات في الاكسون 15و 16 لجين .DGAT1 تم عمل تفريد كهربي لنواتج تفاعل البلمرة المختلفة وعمل تعداد للتتابعات ثم عمل محاذاة لهذه التتابعات الناتجة بإستخدام الادوات .(Clastal Omega and Jalview)

أظهر تحليل ناتج هذا التعداد إلى وجود 3طُرز جينية مختلفة جديدة في العينات محل الدراسة .الطَرز الاول BB :وجد به إثنان من الاختلافات النيوكليتيدية وهما T12Cو G219A والطَرز الثاني DD :به أيضا إثنان من الاختلافات وهما C84Tو G219A بينما إحتوى الطَرز الثالث على إختلاف واحد فقط هو .G219A أظهرت نتائج التحليل الإحصائي الخطي (GLM)أن المجموعة ذات الطَرز الجيني DD كانت أعلى معنويا ($P<0.001$) في محتوى الدهون بينما المجموعة ذات الطَرز الجيني BBسجلت زيادة معنوية ($P<0.01$) في محتوى الجوامد الكلية .من ناحية أخرى لم تظهر المجموعة ذات الطَرز AGأي تأثير معنوي على مكونات اللبن .تم إرسال هذه الاختلافات النيوكليتيدية الجديدة إلى بنك الجينات وتم تسجيلها تحت أرقام *OM418856, OM418857* و *OM418858* حتى يتم نشرها لاحقاً .علاوة على ذلك أظهرت ترجمة نتائج تتابع الجزء محل الدراسة أن الإختلاف النيوكليتيدي G219Aتسبب في إستبدال الحمض الأميني جليسين إلى سيرين في أكسون ١٦ عند الموقع 106في تتابع الأحماض الأمينية.

تم اختبار هذا الاستبدال (G106S) بواسطة الأداة SIFT وأظهرت أنها غير ضارة بقيمة ٠.٤٨ أدى إستبدال الحمض الأميني جليسين ) وهو حمض أميني صغير يقع على سطح جزئ البروتين (إلى سيرين )وهو حمض أميني أكبر من الجليسين (إلى إفتقاد العديد من التفاعلات مع الجزيئات المجاورة في الهيكل النهائي للبروتين.

أظهرت النتائج العديد من التباينات الوراثية في جين DGAT 1 والمرتبطة بمكونات اللبن، لذلك يمكن أن تستخدم كدلائل وراثية في إختيار ماعز اللبن في برامج التربية المستخدمة في نظم تحسين جودة اللبن.