

Bioinformatics Analysis of DGAT 2 Gene in some Animal Species

Vol.4 (4), pp. 51-55, July 2018

ISSN 4372-2608

DOI: <https://doi.org/10.26765/DRJBB.2018.5708>

Article Number: DRJA152025708

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Direct Research Journal of Biology and Biotechnology

<http://directresearchpublisher.org/journal/drjbb>

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Received 29 June 2018; Accepted 25 July, 2018

Diacylglycerol O-acyltransferase (DGAT2) with a molecular weight of 42kDa is a microsomal enzyme that catalyzes the final step of triglyceride synthesis, on milk yield and composition. This present study explained the genetic diversity of DGAT2 gene in selected ruminants, non-ruminants and poultry species. A total of Seventeen sequences of DGAT2 gene from nine species: Cattle (4), Sheep (2), Goat (3), Swine (2), Mouse (3), Rabbit (1), Chicken (1), Turkey (1) and Duck (1) were retrieved from the GenBank (www.ncbi.nlm.nih.gov). Neighbor-Joining Trees was constructed each using P-distance model and pair wise deletion gap/missing data treatment. The reliability of the trees was calculated by bootstrap confidence values, with 1000 bootstrap iterations using MEGA 6.0 Software. Dxy (0.00) between sheep and goat was smaller inferring their closer relationship in evolutionary trend than those of cattle. The maximum Dxy value (0.06) was observed between chicken and turkey while the minimum Dxy

value (0.04) was also seen between chicken and duck and the divergence time displayed the earliest differentiation between them. The minimum Dxy (0.00) was observed between Mouse, Swine and Rabbit respectively. Sequences of DGAT2 genes of sheep and goat clustered closely than those of cattle. Mouse and Rabbit clustered closely than those of Swine whereas sequences of Chicken clustered closely with those of duck than turkey although, the sequences of the poultry species were closer to each other in the evolutionary trend. The clustering result of DGAT2 gene was consistent with the taxonomy in the NCBI. The results of these findings would provide an insight in the understanding of the genetics distance among and within species for selection purposes.

Keywords: Phylogenetic, analysis, DGAT2, divergence, genetics, species

INTRODUCTION

Bioinformatics has become an important part of many areas of biology. In experimental molecular biology, bioinformatics techniques such as image and signal processing allow extraction of useful results from large amounts of raw data. In the field of genetics and genomics, it aids in sequencing and annotating genomes and therefore we can observe polymorphic sites and gene expression; similarities and differences between and within gene sequences in the varied species (Jing-Fen *et al.*, 2008). On the other hands gene mapping research has led to the discovery of many polymorphic sites throughout the ruminants' genome that can serve as genetic markers for selection in breeding schemes (Jing-Fen *et al.*, 2008). Two different DGAT enzymes have been identified, DGAT1 and DGAT2 and in most species, DGAT2 contains 350-400 amino acids and its molecular weight is 42 kDa (Cases *et al.*, 2001). DGAT2 localizes to the endoplasmic reticulum (ER), and is less hydrophobic. DGAT2 has also been found on the surface of cytosolic lipid droplets, mitochondria and mitochondria-associated-

membranes (MAM) that are the specialized domains of the ER associating tightly with mitochondria. This juxtaposition of ER membrane and mitochondrial membranes is thought to channel lipids between ER and mitochondria. The fact that DGAT2 could redistribute around lipid droplets suggested that TG synthesized by DGAT2 could be directed into cytosolic lipid droplets for storage. DGAT2 appears to be more active at lower concentration of substrates (fatty acyl-CoA, 0-50 M), unlike DGAT1 (with higher activity at >100), indicating DGAT2 has a higher affinity for fatty acyl-CoA. This study aimed at examining the DGAT2 genetic analysis of some animal species including some ruminants, non-ruminants and poultry species.

MATERIALS AND METHODS

Sequence sources of DGAT2

A total of Seventeen sequences of DGAT2 from nine

Table 1. Variation in the sequence length of the animal species selected.

Species	(Scientific name)	N	Gene bank accession number	(bp)
Cattle	<i>Bos taurus</i>	4	AJ534379.1, AJ534374.1, AJ53437.1, AJ519786.1	1,999 2,006 2,788 1,914
Sheep	<i>Ovis aries</i>	2	XM_012154872.2, GAAI01007804.1	2,341 2,460
Goat	<i>Capri hircus</i>	3	NM_001314305.1, HM566448.1, XM_018058853.1	1,130 1,133 2,300
Swine	<i>Sus scrofa</i>	2	NM_001160080.1, AY093657.1	1,481 1,935
Mouse	<i>Mus musculus</i>	2	HQ448288.1, AF384160.1	1,266 1,330
Rabbit	<i>Oryctolagus cuniculus</i>	1	XM_008263828.2	2,266
Chicken	<i>Gallus gallus</i>	1	XM_419374.5	2,394
Turkey	<i>Meleagris gallopavo</i>	1	XM_010706269.2	2,232
Duck	<i>Anas platyrhynchos</i>	1	XM_005024101.3	2,457

bp=base pair

species: Cattle (4), Sheep (2), Goat (3), Swine (2), Mouse (3), Rabbit (1), Chicken (1), Turkey (1) and Duck (1) were retrieved from the GenBank (www.ncbi.nlm.nih.gov). The Gen Bank accession numbers of DGAT2 of these species are Cattle (AJ534379.1, AJ534374.1, AJ534371.1, and AJ519786.1), Sheep (XM_012154872.2, GAAI01007804.1), Goat (NM_001314305.1, HM566448.1, and XM_018058853.1), Swine (NM_001160080.1, AY093657.1), Mouse (HQ448288.1, AF384160.1), Rabbit (XM_008263828.2), Chicken (XM_419374.5), Turkey (XM_010706269.2) and Duck (XM_005024101.3).

Sequence alignment, translation and comparison

Sequence alignments, translations and comparisons of DGAT2 gene were done using Clustal W as described by (Larkin *et al.*, 2007) using DNA weight matrix Clustal W (1.6). For both pair wise and multiple alignments, gap opening penalty of 15 and gap extension penalty of 6.66 were used with the transition weight of 0.5 respectively.

Phylogenetic analysis

Neighbor-Joining trees was constructed each using P-distance model and pair wise deletion gap/missing data treatment. The construction was on the basis of genetic distances, depicting phylogenetic relationships among the DGAT nucleotide sequences of the studied species. The reliability of the trees was calculated by bootstrap confidence values (Felsenstein, 1985), with 1000 bootstrap iterations using MEGA 6.0 software (Tamura *et al.*, 2013).

UPGMA tree construction

Unweighted pair group method using arithmetic average

(UPGMA) trees for each gene was constructed with consensus sequences using same model as that of the tree neighbour joining tree. All sequences were trimmed to equal length corresponding to same region before generating the tree.

Average nucleotide per site construction Dxy for heterogeneity and homogeneity

Estimated distance matrix for DGAT2 genes between consensus sequences of 9 species selected was constructed using the distance model of the MEGA 6.0 version.

RESULTS AND DISCUSSION

The sequence length varied from 1,130- 2,788 base pair. The highest coding region were the sequence of *Bos Taurus* (Cattle) with the coding region of 2,788 while the least were the sequence of *Capri hircus* (Goat) with the coding region ranged between 1,130 - 1,133 base pair respectively (Table 1). The average number of nucleotide substitutions per site (Dxy) of the DGAT2 gene between species is shown (Table 2). Dxy is the index of DNA divergence between or among the sequences. The larger the Dxy, the larger the genetic distance. Based on Dxy, a phylogenetic tree was constructed for all the species using the UPGMA method (Figure 2). The dendrogram of different species based on the differentiation of the DGAT2 gene agreed with the taxonomy of NCBI (Figure 1). Evolutionary divergence of DGAT2 revealed that, Dxy (0.21) was realized between Cattle and Goat. Dxy (0.00) between sheep and goat was smaller inferring their closer relationship in evolutionary trend than those of cattle. The results of the present study is in agreement with the report of Ogah *et al.* (2017) and Faith and Owoeye, (2017a) who reported a minimum Dxy value of (0.01) in DGAT1 and lactoferrin gene between sheep and

Table 2. Evolutionary divergence of DGAT2 gene sequences between species selected.

Species	1	2	3	4	5	6	7	8
Cattle								
Sheep	0.05							
Goat	0.21	0.00						
Mouse	1.00	0.01	0.20					
Swine	0.00	0.01	0.01	0.00				
Rabbit	0.00	0.01	0.00	0.00	1.00			
Chicken	0.23	0.00	0.00	0.03	0.00	0.00		
Turkey	0.05	0.00	0.00	0.01	0.00	0.00	0.06	
Duck	0.06	0.00	0.00	0.00	0.00	0.00	0.04	0.17

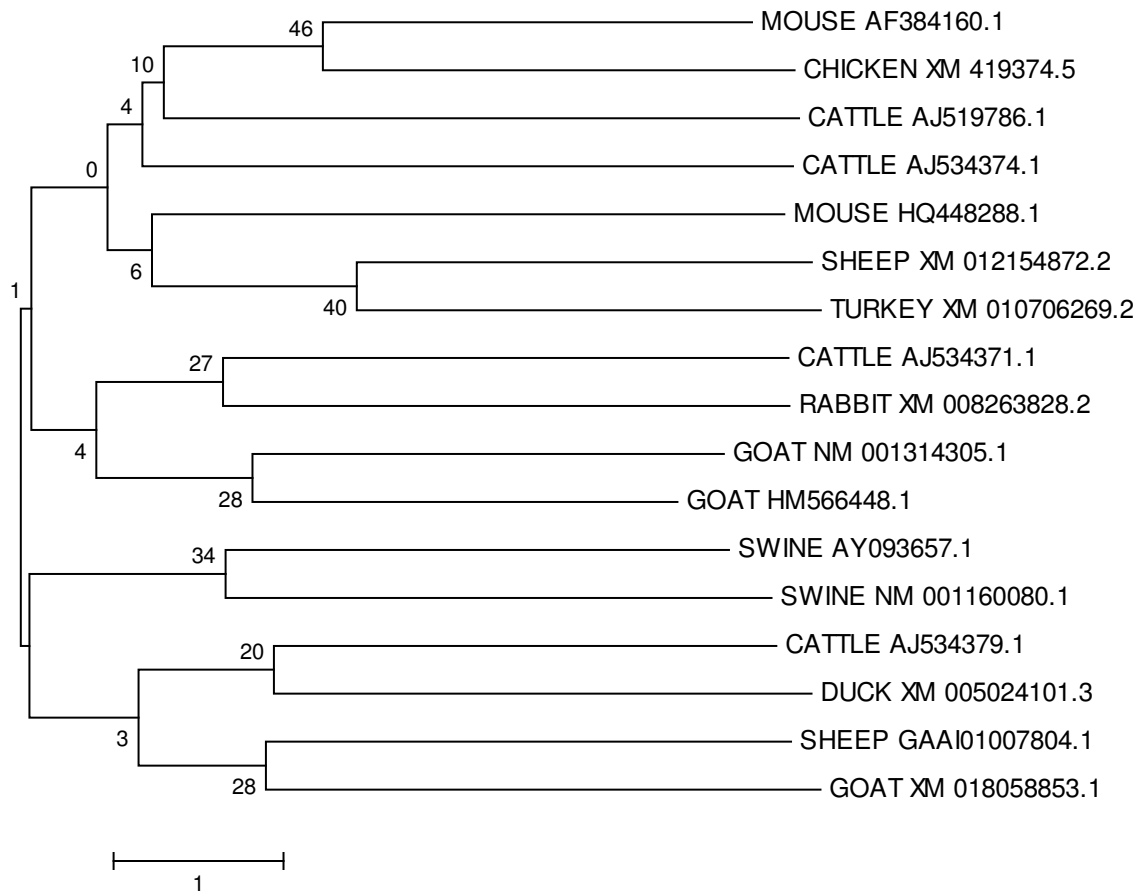


Figure 1. Neighbour joining tree.

goat respectively. Also Yang and Yoder (2003), Yakubu *et al.* (2014), and Faith and Abubakar, (2017b) reported a smallest Dxy (0.00) in ruminant species. The maximum Dxy value (0.06) of DGAT2 gene in poultry species was observed between chicken and turkey where as the minimum Dxy (0.04) was observed between chicken and duck and the divergence time displayed the earliest

differentiation between them. This result revealed that the genetic distance between chicken and turkey was higher as compared to chicken and duck, implying the closer evolutionary relationship between duck and chicken. The neighbour-joining tree clearly revealed that clustering was largely species-wise. The presence of numerous alleles at a particular DGAT2 locus is evidence of the long-term

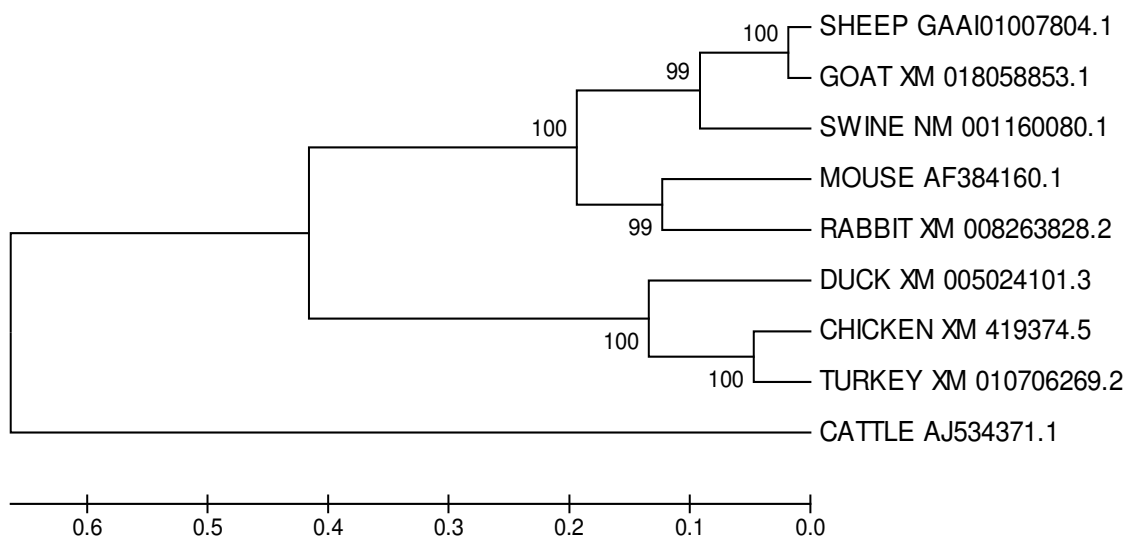


Figure 2. UPGMA tree.

evolutionary persistence of the locus (Yakubu *et al.*, 2014). This is suggested by the fact that the alleles in one species are often more closely related to the alleles in closely related species than to the other alleles in the same species. The species wise clustering might be due to species specific residues (Takahashi and Nei, 2000) and such patterns of the sequences may be explained by gene conversion and balancing selection. UPGMA topology, explained that all the chicken sequences were closer to duck sequences, compared to those of turkey sequence. Sequences of sheep and goat were closer than those of Cattle. The sequences of both mouse and rabbit tend to cluster closely than those of swine. This variation could be explained based on their genetic distance value from each other from evolution.

Conclusion

Studying of DGAT2 genes is of paramount importance in assessing economical traits of animal species. The results of this study explain clearly, the evolutionary and genetic distance between and within species. The genetic distance (Dxy) between species could be smaller and larger, the smaller the distance, the closer the evolutionary relationship and larger the genetic distance (Dxy), the farther the relationship between or within species from evolution. Sequences of DGAT2 genes of sheep and goat clustered closely than those of cattle, mouse and rabbit clustered closely than those of swine whereas sequences of chicken clustered closely with those of duck than turkey and nevertheless, the

sequence of the poultry species studied were closer to each other in the evolutionary trend.

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