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Computational Analysis of the Sequences of LIPE Gene of Selected Ruminants and Non-ruminants

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Authors' contributions

This work was carried out in collaboration among all authors. Author RBF designed the study, performed the prediction and phylogenetic analysis, wrote the protocol and wrote the first and final draft of the manuscript. Author MOA managed the analyses and corrected the first draft of the study. Author OHO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Tropically adapted farm animals are characterized by low meat and milk productivity. Traditionally, mass selection has been widely employed in breeding for improved animal performance. However, improving animal productivity using mass selection is laborious and usually less effective. Advances in molecular techniques such as DNA sequencing analysis provide the opportunity to characterize meat and milk influencing genes, which can lead to faster genetic improvement but often unaffordable and expensive particularly in developing countries. Unlike the wet laboratory analysis, computational molecular analyses is comparatively cheaper in pre-screening of the functional impacts of nonsynonymous single-nucleotide variants of some performance traits-related genes such as hormone-sensitive lipase (LIPE).

A total of fifteen (15) LIPE nucleotide sequences comprising pig (3), cattle (3), water buffalo (2), camel (2), goat (2) and sheep (3) were retrieved from the Genbank. Also, twenty (20) functionally associated genes with the LIPE gene including perilipin 1, 2 & 5 protein kinase cAMP-activated catalytic subunit alpha, protein kinase X-linked were determined using the GeneMANIA.

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Functional analysis of non-synonymous single nucleotide polymorphism using PROVEAN showed that ten amino acid substitutions (S216C, P107del, Q28_P29insRATHVA, S41_S44dup, A640M, S940A, L660I, D86delinsWA, S1000F) in water buffalo and pig (X678E, V789K, G987del, T218K, Q2234del, L278H, Q321del, L1023delinsPKL, P1452V, H1267delinsRFT), nine in sheep (F67_P68delinsVQ, R24G, A247_R248dup, L122L, L144P, S149K, S125S, S224H, G148M) and goats (A450Y, P480H, G490delinsPHQ, L500R, R550del, S100_S101dup, E600S, A700Q, P754delinsQAW) and five in camel (A320A, S210S, L130L, T400T, L440I) were found neutral indicating their beneficial effect while only T110A out of the fifteen amino acid substitutions was found deleterious in cattle. The obtained phylogenetic trees from the nucleotide sequences showed a closer relationship among the members of the Bovidae family particularly the sheep and cattle.
This information may aid future research that aims at the selection of the studied animals for improved meat and milk quality traits.

Keywords: LIPE gene; hormone sensitive lipase; computational analysis; functional analysis; ruminant and non-ruminant.

1. INTRODUCTION

LIPE gene encodes hormone-sensitive lipase (HSL), which is an intracellular neutral lipase that plays a key role in energy mobilization and hydrolyzing of accumulated fats [1]. HSL along with adipose triglyceride lipase and monoacylglycerol lipase synergistically affects fats in adipocyte lipid droplet and breaks down triacylglycerol to non-esterified fatty acids and glycerol. LIPE is commonly expressed in tissues: adipose, skeletal, muscle and organs: adrenal gland, testis, pancreatic b-cells and ovary [2,3]. The hormone-sensitive lipase gene (LIPE) is located on chromosome 19 and has been found to be highly conserved in pigs, humans, mice and rats etc [4]. Altered LIPE function in mice was associated with significant reduction of body fat mass, low levels of circulating fatty acid, adipocyte hypertrophy, lipid-filled macrophages and an increase in basal lipolysis [3]. Also, adipocytes of LIPE null mice were incapable of releasing glycerol with massive accumulation of cellular diacylglycerol [5]. Similarly, the polymorphism of the human LIPE gene was associated with variation in plasma lipid concentrations [6,7]. Hormone-sensitive lipase also plays a fundamental role in the regulation of energy balance by releasing free fatty acids from adipose triacylglycerol stores and triacylglycerol catabolism via a hormone-stimulated lipolysis process. These fatty acids can be subsequently transferred to other body compartments to be oxidized for other biochemical reactions particularly in lactating animals because the synthesis of milk components involves the mobilization of lipid depots to satisfy the large energy demands of the mammary gland. Single Nucleotide Polymorphism in LIPE have been associated with milk yield and milk fat contents of goats [8], carcass and meat quality traits in steers [9], superior carcass and meat quality traits in pigs [10] and intra-muscular fat and fatty acid composition of bovine meat [8]. Since LIPE has direct effects in lipolysis, structural changes on the enzyme are expected to modify the fatty acid composition of tissues which may also affect many other economic traits particularly those that require free fatty acids as metabolic precursors [11,12]. Therefore, this study computationally assessed the attendant functional effects of the genetic variants of the LIPE gene of some ruminants (cattle, sheep and goats) and non-ruminants species (water buffalo, camel and pig) with a view to providing relevant genetic information on their amenability as marker in marker-assisted selection for improved performance of the studied species.

2. MATERIALS AND METHODS

2.1 Data Retrieval

A total of fifteen (15) LIPE nucleotide sequences comprising pig (3), cattle (3), Water buffalo (2), camel (2), goat (2) and sheep (3) were retrieved from the Genbank (https://www.ncbi.nlm.nih.gov/genbank/). The Genbank accession numbers of the sequence were: AH013024.2, AH013025.2, AH013026.2 (pig), AGFL01151421.1, AGFL01151423.1, AY898616.1 (cattle), AY900493.1, JU592870.1 (water buffalo), JDVD01023012.1, JDVD01023013.1 (camel), EU273879.1, JT702894.1 (goat), DQ647327.1, KC585035.1, KC610086.1 (sheep).

2.2 Sequence Alignment and Translation

Sequence alignment, translation and comparison of the retrieved LIPE sequences were done with

ClustalW of MEGA software version 7.0 [13] using gap open penalty of -400 and gap extension penalty of 0.0.

2.3 LIPE and Functionally Similar Genes

The functionally related genes to LIPE were obtained using the GeneMANIA (http://www.genemania.org) [14]. Also, the genetic interactions, pathways, co-expression, co-localization and protein domain similarity of the obtained genes were determined. GeneMANIA finds functionally associated genes and genes that are likely to share function with the query gene using a wealth of genomics and proteomics data.

2.4 Phylogenetic Trees and Evolutionary Distance Analysis

The phylogenetic relationships among the LIPE nucleotide sequences of swine, cattle, water buffalo, camel, goat and sheep was drawn using the complete deletion and p-distance options of the Neighbor-Joining method [15]. The reliability of the tree was estimated by bootstrap confidence values with 750 bootstrap replications [16]. Similarly, the evolutionary distances of the nucleotide sequences of the studied species were computed using the UPGMA method of [13]. The analysis involved 15 nucleotide sequences. Codon positions included were 1^{st}+2^{nd}+3^{rd}+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 4847 positions in the final dataset. Evolutionary analyses were conducted using MEGA 7.0 [17].

2.5 Functional Analysis

Protein Variation Effect Analyser (PROVEAN) was used to predict the functional effect of protein sequence variations including single amino acids substitutions, insertion and deletions [18]. The prediction based on the change caused by a given variation in the similarity of the query sequence to a set of its related protein sequence, variants with a PROVEAN score above -2.5 (the prediction cut off) are considered "NEUTRAL" while variants with PROVEAN score equal to or below -2.5 are considered "DELETERIOUS".

3. RESULTS

In this study LIPE gene was found to have an association with 20 other different genes (Table 1). The genetic interactions, co-expression and protein domain similarity of LIPE with other related genes is shown in Fig. 1. Among the most important ones are the perilipin 1, 2 & 5 genes which play critical roles in the regulation of lipolysis and mobilization of fats in the adipose tissue.

Table 1. Gene description using GeneMANIA

SN	Gene	Description
1	LIPE	lipase E, hormone sensitive type
2	FABP4	fatty acid binding protein
3	PLIN5	perilipin 5
4	PRKACA	protein kinase cAMP-activated catalytic subunit alpha
5	PLIN1	perilipin 1
6	PRKAR1A	protein kinase cAMP-dependent type I regulatory subunit alpha
7	PRKX	protein kinase, X-linked
8	PLIN2	perilipin 2
9	NCEH1	neutral cholesterol ester hydrolase 1
10	PRKAR2A	protein kinase cAMP-dependent type II regulatory subunit alpha
11	PRKAR2B	protein kinase cAMP-dependent type II regulatory subunit beta
12	PRKACG	protein kinase cAMP-activated catalytic subunit gamma
13	PRKACB	protein kinase cAMP-activated catalytic subunit beta
14	AADACL2	arylacetamide deacetylase like 2
15	AADAC	arylacetamide deacetylase
16	AADACL4	arylacetamide deacetylase like 4
17	AADACL3	arylacetamide deacetylase like 3
18	NSFL1C	NSFL1 cofactor
19	AFMID	Arylformamidase
20	GPD1	glycerol-3-phosphate dehydrogenase 1
21	PLIN3	perilipin 3

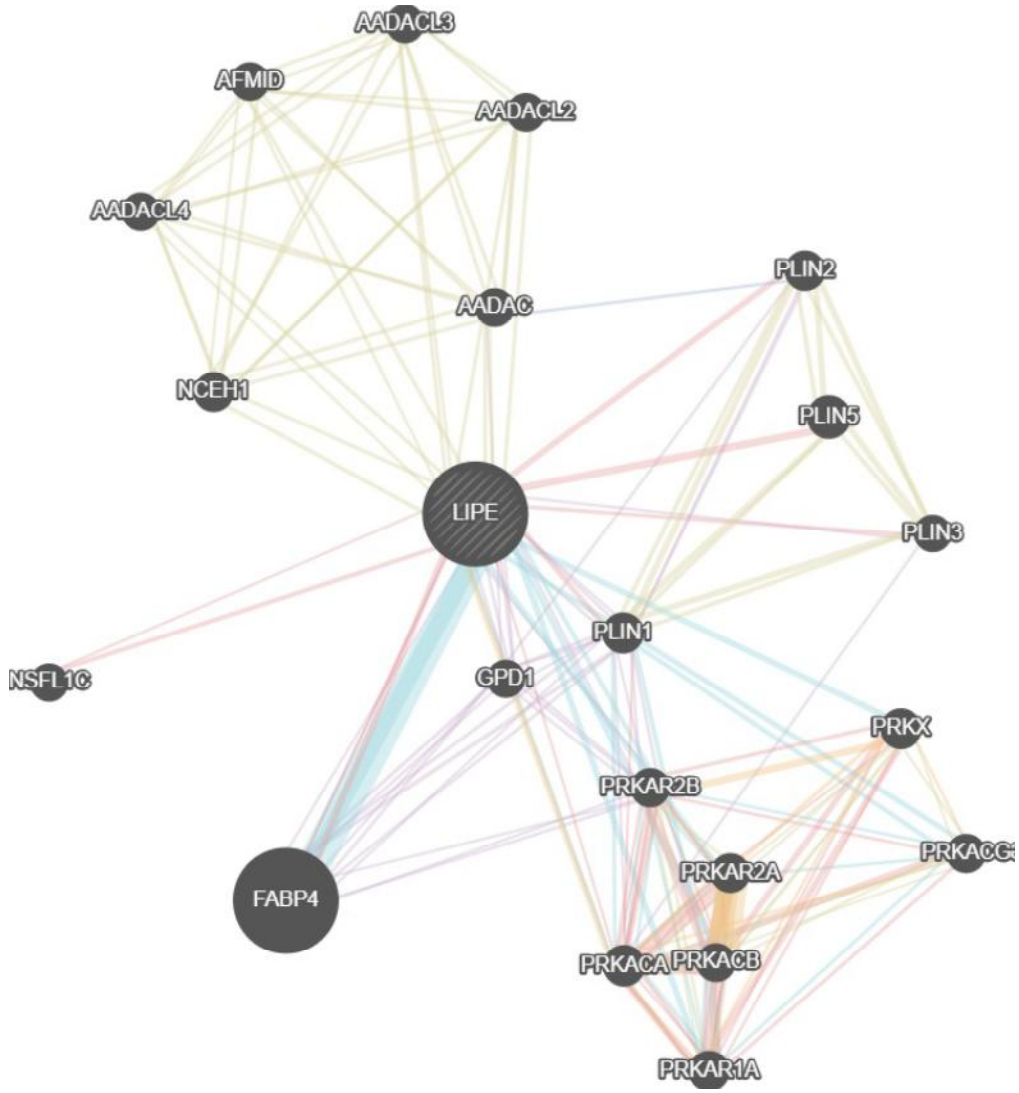

Fig. 1. GeneMANIA derived functional association between LIPE and other related genes

Out of the fifteen (15) amino acid substitutions in each of the studied species, five were deleterious in water buffalo (Table 2) and pig (Table 6), six in goat (Table 5) and sheep (Table 7), ten in camel (Table 3) and one in cattle (Table 4) while the remaining 10, 9, 5 and 14 amino acid substitutions were neutral for water buffalo and pig, goat and sheep, camel and cattle respectively. The phylogenetic tree obtained from the consensus sequences of the studied species using UPGMA method showed a closer relationship between cattle and sheep than water buffalo and goat at this locus.

Table 2. Functional analysis of coding nsSNP of the LIPE gene of water buffalo (*Bubalus bubalis*) using PROVEAN

Variant	PROVEAN score	Prediction
S216C	-1.032	Neutral
P107del	0.022	Neutral
Q28_P29insRATHVA	-0.534	Neutral
Q93_R94insRWTYV	-2.900	Deleterious
S41_S44dup	-1.525	Neutral
L81_M85delinsISYP	-2.812	Deleterious
A640M	-0.021	Neutral
S940A	-0.100	Neutral
C600W	-6.186	Deleterious
A640M	-0.021	Neutral
L660I	-0.916	Neutral
C600W	-6.186	Deleterious
D86delinsWA	-1.688	Neutral
G620Y	-8.264	Deleterious
S1000F	0.000	Neutral

A = Alanine; C = Cysteine; D = Aspartic acid; F = Phenylalanine; G = Glycine; H = Histidine, I = Isoleucine; L = Leucine; M = Methionine; P = Proline; Q = glutamine; R = Arginine; S = Serine; T = Threonine; V= Valine; W = Tryptophan; Y = Tyrosine

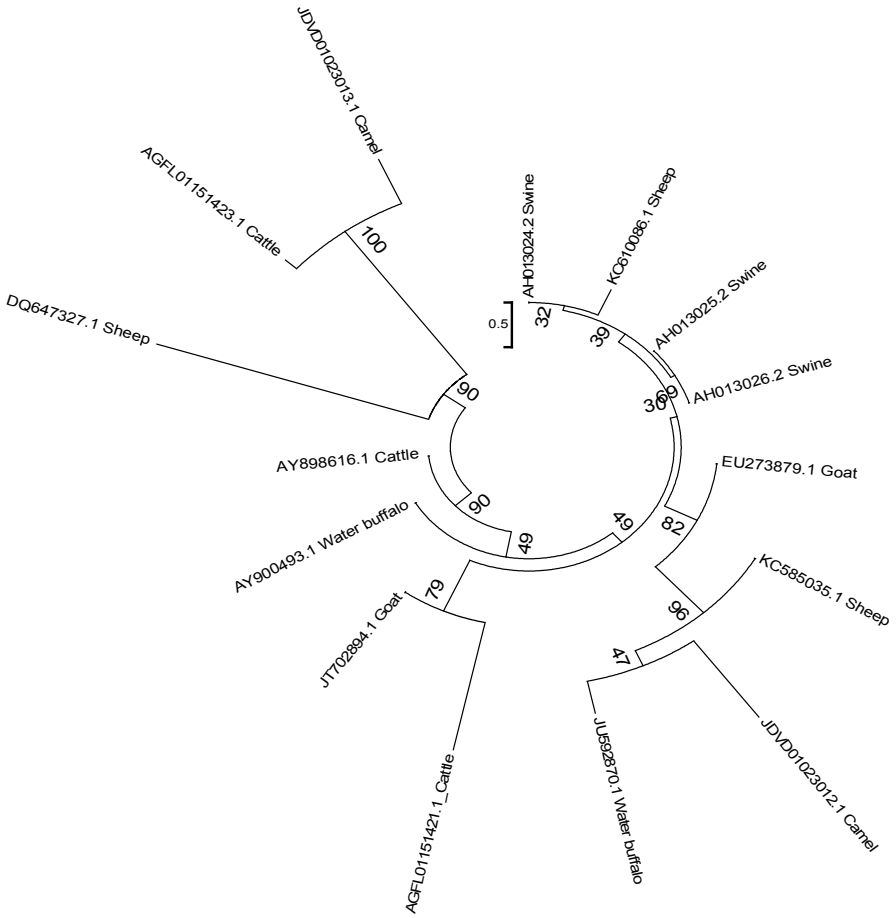

Fig. 2. Neighbour-joining tree obtained from LIPE gene in some ruminants and non- ruminants using the neighbor joining method

Table 3. Functional analysis of coding nsSNP of the LIPE gene of camel (*Camelus dromedarius*) using PROVEAN

Variant	PROVEAN score	Prediction
C50del	-19.000	Deleterious
A480S	-3.000	Deleterious
V500R	-7.000	Deleterious
L440del	-14.000	Deleterious
A320C	0.000	Neutral
G350_P351insHY	-11.000	Deleterious
S210A	0.000	Neutral
L130G	0.000	Neutral
T400R	0.000	Neutral
P420Y	-10.000	Deleterious
L440I	-2.000	Neutral
S460_A461insWQA	-12.000	Deleterious
R470T	-6.000	Deleterious
P490_A491insFRS	-12.000	Deleterious
V340Y	-5.000	Deleterious

A = Alanine; C = Cysteine; F = Phenylalanine; G = Glycine; H = Histidine, I = Isoleucine; L = Leucine; P = Proline; Q = Glutamine; R = Arginine; S = Serine; T = Threonine; V= Valine; W = Tryptophan; Y = Tyrosine

Table 4. Functional analysis of coding nsSNP of the LIPE gene of cattle (*Bos indicus*) using PROVEAN

Variant	PROVEAN score	Prediction
T110A	-2.500	Deleterious
G180del	0.000	Neutral
E360_S361delinsTRE	0.000	Neutral
A370delinsTR	0.000	Neutral
T110W	3.500	Neutral
W380_S381delinsGIP	0.000	Neutral
A420_L421delinsTATTT	0.000	Neutral
Q650del	0.000	Neutral
L754C	0.000	Neutral
K1000_X1001dup	0.000	Neutral
V680_P681dup	0.000	Neutral
F100G	5.000	Neutral
L754T	0.000	Neutral
R390delinsPKL	0.000	Neutral
A901P	0.000	Neutral

A = Alanine; C = Cysteine; E = Glutamic acid; F = Phenylalanine; G = Glycine; I = Isoleucine; L = Leucine; K = Lysine; P = Proline; Q = Glutamine; R = Arginine; S = Serine; T = Threonine; V = Valine; W = Tryptophan

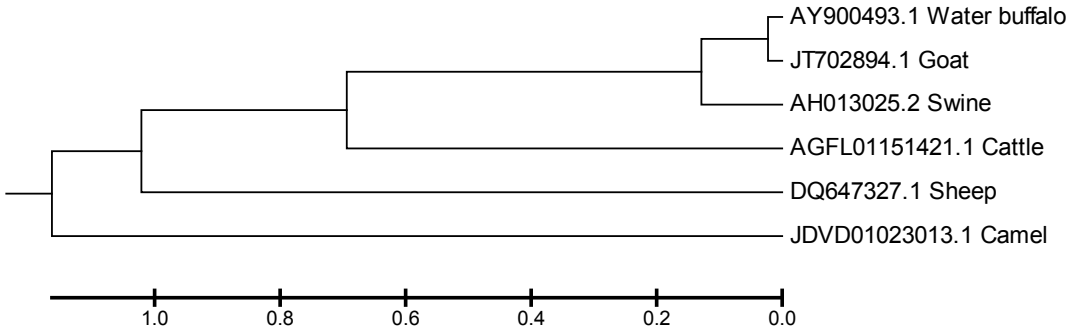

Fig. 3. Phylogenetic tree derived from selected sequences of LIPE gene in some ruminants and non-ruminants species using the UPGMA method

Table 5. Functional analysis of coding nsSNP of the LIPE gene of goat (*Capra hircus*) using PROVEAN

Variant	PROVEAN score	Prediction
F250delinsVAS	-13.488	Deleterious
A450Y	0.205	Neutral
W460delinsQWA	-2.673	Deleterious
E220_G221insHGL	-4.178	Deleterious
P480H	-1.023	Neutral
G490delinsPHQ	-2.394	Neutral
L500R	-0.482	Neutral
R550del	-0.410	Neutral
S100_S101dup	-1.534	Neutral
R110_L111insGR	-4.434	Deleterious
Q120_A121delinsDQ	-3.733	Deleterious
E600S	-0.040	Neutral
A700Q	-0.444	Neutral
P754delinsQAW	-0.222	Neutral
S470del	-5.080	Deleterious

A = Alanine; D = Aspartic acid; E = Glutamic acid; F = Phenylalanine; G = Glycine; H = Histidine; L = Leucine; P = Proline; Q = Glutamine; R = Arginine; S = Serine; W = Tryptophan; Y = Tyrosine

Table 6. Functional analysis of coding nsSNP of the LIPE gene of pig (*Sus scrofa*) using PROVEAN

Variant	PROVEAN score	Prediction
X678E	-0.286	Neutral
V789K	-0.378	Neutral
G987del	0.388	Neutral
L465del	-5.219	Deleterious
L470del	-3.844	Deleterious
T218K	0.200	Neutral
Q2234del	0.667	Neutral
P458delinsQAS	-5.374	Deleterious
G473delinsWS	-3.089	Deleterious
L278H	-0.150	Neutral
Q321del	0.025	Neutral
S738delinsNN	-3.361	Deleterious
L1023delinsPKL	0.443	Neutral
P1452V	0.000	Neutral
H1267delinsRFT	0.000	Neutral

A = Alanine; E = Glutamic acid; F = Phenylalanine; G = Glycine; H = Histidine; K = Lysine; L = Leucine; N = Asparagine; P = Proline; Q = Glutamine; R = Arginine; S = Serine; T = Threonine; V= Valine; W = Tryptophan

4. DISCUSSION

The hormone-sensitive lipase (encoded by the LIPE gene) is an intracellular enzyme that plays a fundamental role in the regulation of energy balance by releasing free fatty acids from adipose triacylglycerol stores. LIPE gene had some genetic interactions, co-expression and protein domain similarity with fatty acid binding protein, perilipin 1, 2 & 5, protein kinase cAMP-activated catalytic subunit alpha, protein kinase cAMP-dependent type I regulatory subunit alpha, protein kinase X-linked, neutral cholesterol ester hydrolase 1, protein kinase cAMP-dependent type II regulatory subunit alpha, protein kinase cAMP-dependent type II regulatory subunit beta and ten other genes (Fig. 1). The present findings showed that the LIPE gene of pig, cattle, water buffalo, camel, goat and sheep is polymorphic in nature and earlier studies have associated polymorphism of the LIPE gene with superior milk yield and milk fat contents, carcass characteristics including muscle thin, fat thickness of back, intra-muscular fat in different species etc [8,9,10,19]. More so, the amino acid substitution revealed both neutral and

Table 7. Functional analysis of coding nsSNP of the LIPE gene of sheep (*Ovis aries*) using PROVEAN

Variant	PROVEAN score	Prediction
F67_P68delinsVQ	5.232	Neutral
T98_T99delinsWA	-6.142	Deleterious
P116_S117dup	-9.083	Deleterious
L138_N139dup	-9.614	Deleterious
G91del	-9.548	Deleterious
R24G	-2.408	Neutral
A247_R248dup	2.373	Neutral
L122L	0.000	Neutral
E133delinsLN	-12.145	Deleterious
L144P	-1.464	Neutral
S149K	-2.031	Neutral
F151delinsPS	-17.018	Deleterious
S125S	0.000	Neutral
S224H	0.417	Neutral
G148M	-2.323	Neutral

A = Alanine; E = Glutamic acid; F = Phenylalanine; G = Glycine; H = Histidine; K = Lysine; L = Leucine; M = Methionine; N = Asparagine; P = Proline; Q = Glutamine; R = Arginine; S = Serine; T = Threonine; V= Valine; W = Tryptophan

deleterious variants of LIPE gene in all the six studied species. Out of the fifteen (15) amino acid substitutions in all the six studied species, five were deleterious in water buffalo and pig, six in goat and sheep, ten in camel and one in cattle while the remaining 10, 9, 5 and 14 amino acid substitutions were neutral for water buffalo and pig, goat and sheep, camel and cattle respectively. The amino acid substitution that returned neutral indicates that the substitution did not have any damaging effect on the structure and function of the protein while those deleterious amino acid substitution predictions were harmful and could cause an alteration or impairment of protein functions which may lead to impair energy balance, low levels of circulating fatty acid, adipocyte hypertrophy, lipid-filled macrophages and an increase in basal lipolysis particularly in lactating animals [20,21,22]. This is because the synthesis of milk components involves the mobilization of lipid depots to satisfy the large energy demands of the mammary gland [8]. The phylogenetic tree obtained based on the nucleotide sequences of the studied species suggests that goat was more related with water buffalo. Cattle, sheep and swine were more closely related while camel was farther apart at this locus. These observations agree with the submissions of [21,23]. The authors reported similar clustering of cattle, sheep and swine at BMP15 and PAPPA2 loci respectively. Earlier authors [24,25] attributed consistent clustering of sheep and cattle to their classical classification as members of the Bovidae family and to the fact that pig shares the order Artiodactyla with the ruminants. The varying substitutions of amino acids within and across species might be as a result of separate divergence from their common ancestor. This agrees with earlier submissions of [26,27] that as orthologs diverge from their most recent common ancestor, their different evolutionary trajectories lead to divergence in the selective constraints on homologous sites. This information may aid selection of the studied animals for improved milk yield as well as superior meat and milk quality traits particularly in developing country like Nigeria [23].

5. CONCLUSION

The result obtained in this study revealed that LIPE gene is highly polymorphic in the studied ruminant and non-ruminants. Both the deleterious and beneficial mutations were found at LIPE locus. The information emanating from this study would be helpful in performing further study aiming at improving economically important traits such as meat and milk in the studied animal species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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