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Insilico Analysis of Myostatin Gene in Selected Poultry Species

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Authors' contributions

This work was carried out in collaboration among all authors. Author MKE 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 HOO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Myostatin gene (GDF8) is a member of transforming growth-β superfamily and has been reported to act as a negative regular of skeletal muscle during myogenesis, regulation of adipocyte function in livestock species. This study was carried out to computationally investigate molecular genetic variation and categorize precise mutation in myostation gene in selected poultry species at the studied locus. A total of twenty (20) myostatin nucleotide sequences consisting of chicken (12), quail (4) and turkey (4) were retrieved from the GenBank. Functional analysis of nsSNP using PROVEAN showed three amino acid substitutions (P20Q, Y11F and G3R) in chicken, one in quail (Y100R) and three in turkey (N65P, F155W and K95A) were all returned neutral, suggesting their beneficial impacts. The information from nucleotides sequences showed the interclustering and close relatedness of members of phasianidae family (chicken, quail and turkey).

Keywords: Myostatin gene; poultry species; mutation; insilico analysis; sequences.

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1. INTRODUCTION

One of the growth influencing genes affecting traits of economic importance is myostatin gene which leads to increase muscle phenotype in livestock species. Myostatin commonly called growth and differentiation factor 8 (GDF8) is a member of transforming growth factor- β superfamily which is synthesized by a 376 amino acid precursor protein including three domain namely: a C-terminal domain or active molecule, an N-terminal polypeptide domain and a signal sequence [1]. Variations in this gene have been found to be associated with the "double muscle" phenotype in various species of animals like mice [1], cattle [2], Pigs [3] and Sheep [4].' Mutations in myostatin regulatory regions have been shown to be associated with growth and carcass traits in poultry species [5]. Significantly, myostatin gene plays a pivotal during the process of myogenesis in poultry species, it is therefore imperative to illustrate its evolutionary process, mutation effects on the selected poultry species.

Wet laboratory studies to illustrate mutation in a particular gene of interest is laborious, expensive and time-consuming. Computational study can efficiently produce useful information to rationalize and undertake further experimental studies [6-8]. Therefore, computational predictions have become essential for evaluating the disease-related impact of nonsynonymous single-nucleotide variants discovered in exome sequencing [9].

Computational tool such as PROVEAN is more useful for pin-pointing impact analysis of point mutation linking the gene function or gene product [8,10-15]. Consequently, the combination of a different algorithm will improve the accuracy of results or predicted effects of particular mutation [16]. SNPs with amino acid (aa) substitutions can distort protein folding and its stability, leading to altered protein function and protein- interaction including it expression [17-20]. Thus, it would be of interest to identify each mutation in myostatine gene that altered its function resulting in muscle growth in economically important poultry species.

Therefore, the objective of this study is to computationally investigate molecular genetic variation and categorize precise mutation of myostatin gene in the selected poultry species

2. MATERIALS AND METHOD

2.1 Sequence of Poultry Species

A total of twenty (20) MSTN nucleotide sequences comprising of chicken (12), quail (4) and turkey (4) were retrieved from the database of NCBI (www.cbinlm.nih.gov). The Genbank accession numbers of the sequences are GU181328.1, GU181327:1, GU181326.1, GU181320.1, GU181322.1, GU181321.1, GU181325.1, GU181324.1, JN675404.2, JN675405.1, JN675402.1 and JN675403.1 (chicken); KF721293.1, KF721291.1, KF721292.1 and KF721290.1 (Quail); KF721289.1, KF721288.1, KF721287.1 and KF721286.1 (Turkey).

2.2 Sequence Alignment and Translation

Sequence alignment, translation and comparison of the MNSTN gene of the various poultry species were carried out with muscle using mega software version 7 [21] using gap open penalty of -2.9, gap extension penalty of 0.0 and hydrophobicity multiplier of 1.2.

2.3 Functional Analysis

Insilico functional analysis of missense mutations was obtained using (PROVEAN) with threshold value of -2.5 in order to predict functional effect of amino acids substitution as deleterious and neutral of small insertions and deletions. Any protein sequence variants having a PROVEAN score below the threshold value of -2.5 are termed DELETERIOUS and a PROVEAN score above the same threshold value are considered NEUTRAL.

2.4 Evolutionary Comparison

Evolutionary pathway of nucleotide sequence of (Chicken, quail and turkey) were estimated using pairwise methods and p-distance model with bootstrap of 1000 replicate. The analysis involve twenty (20) nucleotides sequences. All positive containing gaps and missing data were eliminated. There were 207 positions in the final dataset using MEGA 7 software [21]

2.5 Tajima's Test of Neutrality

The Tajima's test statistics [22] was estimated. All the positions containing gaps and missing data were eliminated from the dataset. (Complete deletion option) using MEGA 7 software [21]

2.6 Phylogeny Tree Construction

Neighbor-Joining (NJ) trees were constructed using Maximum Composite Likelihood method and pairwise deletion gap/missing data treatment as described by [22] and [23]. The construction was done on the basis of genetic distance, showing phylogenetic relationships among the MSTN nucleotide of the studied species. The reliability of the trees was estimated by bootstrap confidence values (11) with 1000 bootstrap iterations using MEGA 7.0 software [21]. Significantly, UPGMA trees for the MSTN gene were constructed with consensus nucleotide sequences

3. RESULTS

The variation in sequence length is base pair (bp) of MSTN gene within and among the selected poultry species ranges between 228 bp and 602 bp as shown in Table 1.

In the selected species, the lowest bp of 228 and the highest bp of 602 were found in both quail and turkey. The sequences of both quail and turkey had similar bp of 228, 305, 459 and 602 respectively. In chicken, 2, 3, 3 and 4 sequences have similar bp of 374, 373, 381 and 507.

The results of functional analysis of coding non synonymous single nucleotide polymorphism (nsSNPs) of MSTN gene for chicken, quail and turkey are presented in Tables 2-4 respectively.

Twelve amino acid substitutions of chicken were obtained from the alignment of deduced amino acid sequences of chicken. Out of these, nine animo acid substitutions (G9L, D85M, PI6A, L99, F10V, N60A, L50C, S2ST and Y46H) were identified deleterious indicating that the substitutions did impair protein function while the remaining three amino acid substitution, (P2OQ, Y11F and G3R) were identified neutral indicating that the substitutions were predicted to be beneficial. (Table 2), in quail, nine amino acid substitutions (R149_ D150delinsKM, R2A, T10C, Q36V, S135P, M1H, L50V, V10del and K50_C5linsAE) were deleterious indicating that they were predicted to be harmful while (Y100R) was returned neutral indicating that it was predicted to be beneficial. (Table 3). Additionally, ten amino acids variants were obtained from amino acid sequences of turkey, out these, seven (I50, N51delinsGH, Q55del, G60A, L20del, D100F, K75del and E160_D16linsAMP) appeared to be harmful while the remaining three (N65P, F155W and K95A) appeared beneficial.

Table 1. Accession number, base pair and sequence length variation of myostatin gene of chicken, quail and turkey

Selected spp	Accession number	Base pair	Sequence length variation
Chicken	GU181328	381	373-507
	GU181327	381	
	GU181326	381	
	GU181320	373	
	GU181322	373	
	GU181321	373	
	Gu181325	374	
	GU181324	374	
	JN675404	507	
	JN675403	507	
	JN675405	507	
	JN675402	507	
Quail	KF721293	228	228-602
	KF721291	459	
	KF721292	305	
	KF721290	602	
Turkey	KF721289	228	228-602
	KF721288	305	
	KF721287	459	
	KF721286	602	

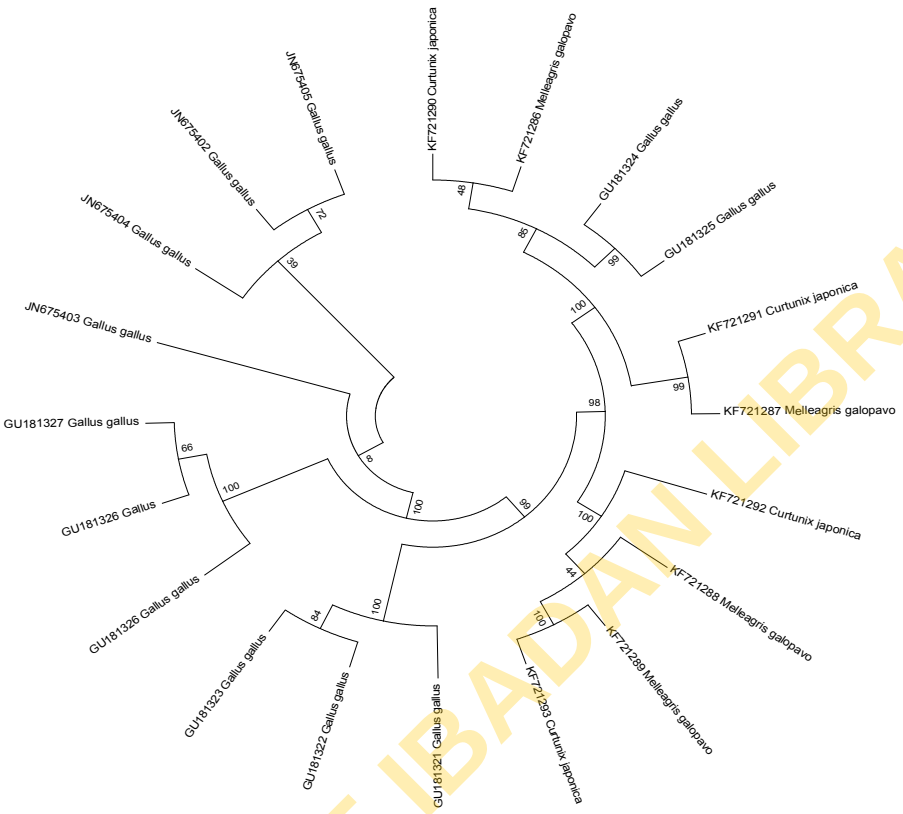

Fig. 1. Phylogenetic relationships of chicken, quail and turkey MSTN nucleotide sequences

The Tajima's neutrality test indicated that, all the species selected showed positive value of D with chicken having the highest value of 3.104806. (Table 5).

The phylogenetic analysis on the basis of nucleotide sequences of MSTN gene as shown in Figs. 1 and 2 depict the interclustering of sequences in the selected poultry species with high level of intermingling between them. The evolutionary pathway and the genetic relationship of MSTN of the studied species with the use of UPGMA (unweighted pair-group method using an arithmetic average) indicated that members of the Phasianidae family were closely related (Fig. 3).

Table 2. Function analysis of coding nsSNP of myostatin gene of chicken using PROVEAN

Variance(12)*	Provean score	Prediction (Cutoff-2.5)
G9L	-9.739	Deleterious
D85M	-5.810	Deleterious
P16A	-7.790	Deleterious
L99K	-4.286	Deleterious
P20Q	-1.687	Neutral
Y11F	-0.596	Neutral
G3R	-1.974	Neutral
F10V	-6.178	Deleterious
N60A	-8.000	Deleterious
L50C	-5.000	Deleterious
S25T	-3.000	Deleterious
Y46T	-5.000	Deleterious

G = Glycine, L=Leucine, D=Aspartic Acid, M= Methionine, P = Proline, A=Alanine, K = Lysine, Q = Glutamine, Y = Tyrosine, F = Phenyalanine, R = Arginine, V=Valine, N = Asparagine, C = Cysteine, S = Serine, T= Threonine, H= Histidine

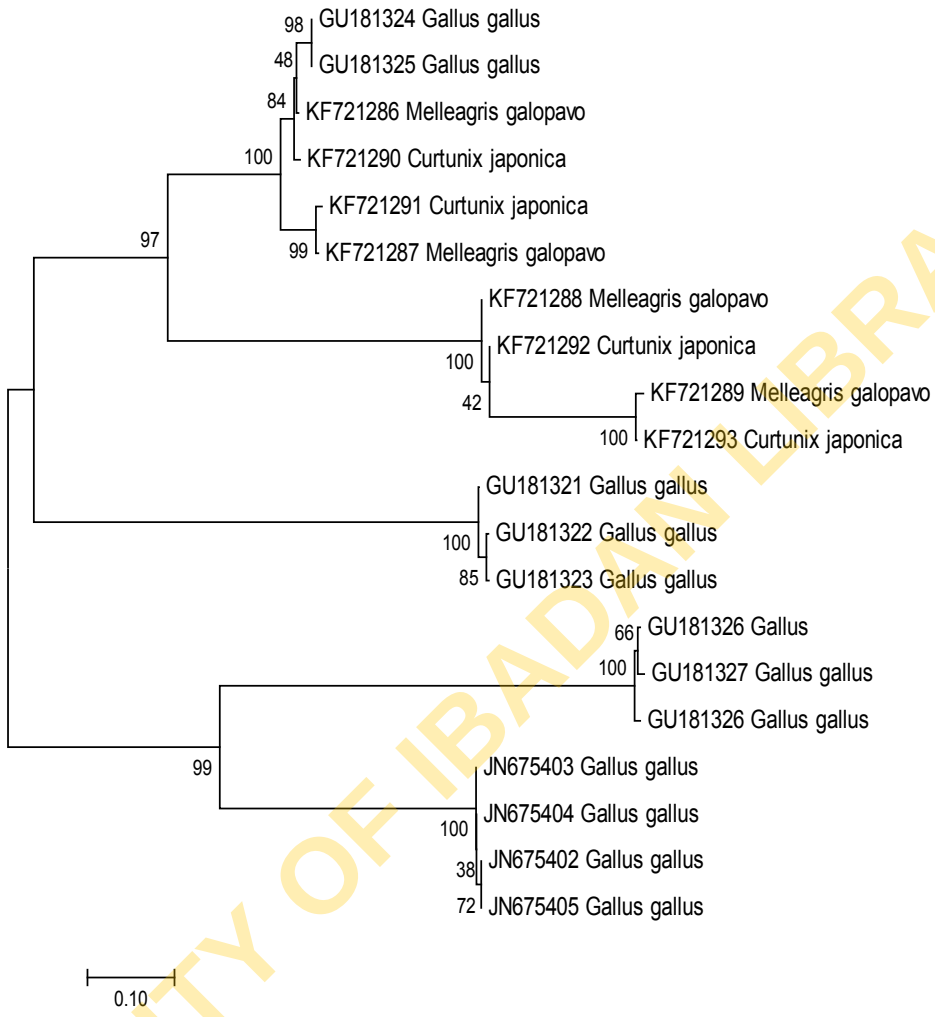

Fig. 2. Phylogenetic tree of chicken, quail and turkey MSTN consensus nucleotide sequences using UPGMA

Table 3. Functional analysis of coding nsSNP of myostatin gene of quail using PROVEAN

Variance(10)*	Provean score	Prediction (Cutoff-2.5)
R149_D150delinsKM	-5.009	Deleterious
R2A	-6.000	Deleterious
T10C	-6.000	Deleterious
Q36V	-7.000	Deleterious
S135P	-5.000	Deleterious
M1H	-7.000	Deleterious
L50V	-3.000	Deleterious
Y100R	-0.389	Neutral
V10del	-2.772	Deleterious
K50_C51insAE	-5.585	Deleterious

R = Arginine, D = Aspartic acid, K = Lysine, M = Methionine, A = Alanine, T = Threonine, C = Cysteine Q = Glutamine, V = Valine, S = Serine, P = Proline, H = Histidine, L = Leucine, Y = Tyrosine, E = Glutamic acid

Table 4. Functional analysis of coding nsSNP of myostatin gene of turkey using PROVEAN

Variance(10)*	Provean score	Prediction (Cutoff-2.5)
I50_N51delinsGH	-13.000	Deleterious
Q55del	-15.000	Deleterious
G60A	-6.000	Deleterious
N65P	-1.704	Neutral
L20del	-5.500	Deleterious
D100F	-4.000	Deleterious
K75del	-15.000	Deleterious
F155W	-0.866	Neutral
K95A	-1.210	Neutral
E160_D16linsAMP	-7.238	Deleterious

I = Isoeucine, N = Asparagine, G= Glycine, H = Histidine, Q=Glutamine, A=Alanine, P = Proline, L = Leucine, D= Aspartic acid, F = Phenyalanine, K = Lysine, E=Glutamic acid, W = Tryptophan, M = Methonine

Table 5. Tajima's test of neutrality of chicken, quail and turkey

Selected species	M	S	Ps	θ	π	D
Chicken	12	296	0.899696	0.297925	0.494289	3.104806
Quail	4	34	0.149123	0.081340	0.099415	2.301847
Turkey	4	3	0.017045	0.009298	0.010417	1.089763

M=number of sequences, S = number of segregating sites,
$\theta = {}^{Ps}/_{a1}$ π= Nucleotide diversity D = Tajima test statistic

4. DISCUSSION

MSTN gene equally known as GDF8, acts as a negative regulator of muscle growth and plays a pivotal role in myogenesis. The short sequence length variation observed in the studied species might be as a result of differences in the genomic region where the sequences were retrieved from and differences due to both complete and partial coding sequences (CDS or PCDS). This is in accordance with the findings of [24] who observed similar results with BMP15 gene in ruminants and non-ruminants animals. The variation in sequence length within and among species might result from evolution and differentiation [25] and this variability might also result from DNA duplication, DNA rearrangement, short tandem repeats (STRs), insertions or deletion of sequences [26]. The variability might initiate unique structures between individual members in conferring different biological activities [27].

In molecular biology, the major concern relating to nsSNPs and population genetics is to identify and characterise the nsSNP, that are functionally related from those that are not [7]. In order to investigate structural and functional impact of amino acid present in coding region of MSTN gene, functional analysis using PROVEAN was performed in which deleterious and neutral effects of amino acid variants were predicted in the studied species. PROVEAN (Protein Variation Effect Analyser) is a bioinformatics tool that predicts if an amino acid substitution or indel has an impact on the biological function of a protein. The deleterious amino acid substitutions in the studied species might result in amino acid change thereby altering protein function which may lead to susceptibility of disease. It may also leads to enzyme activity modification, destabilization of protein structure, disruption of protein interactions and abnormal regulation of muscle mass in the selected species. However, the neutral effect of amino acid substitutions stabilizes the structural integrity of protein, It will also aid in selection and positive genetic modification in the selected species. Prediction of SNPs status is promising in modern genetic analysis and breeding programme as they have been used to identify those animals with higher breeding value [28]. A positive Tajima's D in the selected species indicates low levels of low and high frequency polymorphism signifying a balance selection [29]. This might aid in purifying selection against non-synonymous mutations due to their deleterious impacts.

Chicken-------------------------NPFLEVRVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFL------
QKYPHTHLVHQANPRGSPGPCCT-----------PTKMSPINMLY--------------------------------------FNGKKQIIYGKIPAMVVDRCGCS*---------------
----

NPFLEVRVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKKYKANYCSGECEFVFL------
QKYPHTHLVHQANPKGSAGPCCT-----------PTKMSPINMLY--------------------------------------FNGKEQIIYGKIPAMVVDRCGGS*---------------
------

Chicken-------------------------NPFLEVRVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFL------
QKYPHTHLVHQANPRGSAGPCCT-----------PTKMSPINMLY--------------------------------------FNGKEQIIYGKIPAMVVDRCGGS*---------------
------

Chicken-----------------------MQKLAVYVYIYLFMQIAVDPVALDGSSQPTENAEKDGL----CNACTWRQNTKSSRIEA-IKIQI---------------
LSKLRLEQAPNISRDVIKQ---------LLPKAPPLQELI---------------------------------------DQYDVQRDDSSDGSLEDDDYHAT----------
TETIITMPTE?-

Chicken-----------------------MQKLAVYVYIYLFMQIAVDPVALDGSSQPTENAEKDGL----CNACTWRQNTKSSRIEA-IKIQI---------------
LSKLRLEQAPNISRDVIKQ---------LLPKAPPLQELI---------------------------------------DQYDVQRDDSSDGSLEDDDYHAT----------
TETIITMPTE?-

Chicken-----------------------MQKLAVYVYIYLFMQIAVDPVALDGSSQPTENAEKDGL----CNACTWRQNTKSSRIEA-IKIQI---------------
LSKLRLEQAPNISRDVIKQ---------LLPKAPPLQELI---------------------------------------DQYDVQRDDSSDGSLEDDDYHAT----------
TETIITMPTE?-

Chicken-----------------------------LIFLYKWRE-NQNVASLSLALKYNITK**R-------HNYGYT*GKSKNLQRC-
LCRS*DSLSP*KTVQDILEFDL*NLT*TQALVSGRVLM*RQCCKIGSNSLNPI*ASK*KLL---------------------------------------
MRLDEILLSHSQDRVKMD?-------------------------

Chicken-----------------------------LIFLYKWRE-NQNVASLSLALKYNITK**R-------HNYGYI*GKSKNLQRC-
LCRS*DSLSP*KTVQDILEFDL*NLT*TQALVSGRVLM*RQCCKIGSNSLNPI*ASK*KLL---------------------------------------
MRLDEILLSHSQDRVKMD?-------------------------

Chicken SPTMRSVYI*E**CCLPALLPIVLGNISFQYVKTYPLRSHQINQFTLGCNQMLSSDL*STGL-----*PLTARFIVGTTTNRR-
F*RHEPNQSWS------IKAPLAYIRHTSVASRLSDCICC-----------PGSV*N*KKRGK-------------------------------------
GGGGEKRQKGAVTCKKSCKS*QFMCIFTCSCRSRLIRWLWMAVVS

Chicken SPTMRSVYI*E**CCLPALLPIVLGNISFQYVKTYPLRSHQINQFTLGCNQMLSSDL*STGL-----*PLTARFIVGTTTNRR-
F*RHEPNQSWS------IKAPLAYIRHTSVASRLSDCICC-----------PGSV*N*KKRGK-------------------------------------
GGGGEKRQKAAVTCKKSCKSYAV*FIFTCSCRSRLIRWLWMAVVS

Chicken SPTMRSVYI*E**CCLQALLPIVVGNISFQYVKTYPLRSHQINQFTLGCNQMLSSDL*STGL-----*PLTARFIVGTTTNRR-
F*RHEPNQSWS------IKAPLAYIRHTSVASRLSDCICC-----------PGSV*N*KKRGK-------------------------------------
GGGGEKRQKAAVTCKKSCKSYAV*FIFTCSCRSRLIRWLWMAVVS

Chicken SPTMRSVYI*E**CCLQALLPIVVGNISFQYVKTYPLRSHQINQFTLGCNQMLSSDL*STGL-----*PLTARFIVGTTTNRR-
F*RHEPNQSWS------IKAPLAYIRHTSVASRLSDCICC-----------PGSV*N*KKRGK-------------------------------------
GGGGEKRQKGAVTCKKSCKS*QFMCIFTCSCRSRLIRWLWMAVVS

Chicken -----------------------ST*H*QGRY*AAFTQ-SSSTAGTD*SV*CPEGRQ*RWL----FGR**LSCHNRNDYHNA-YGVTH------------------------
---F*RSELQIHQNGPAEIL---------------------------

Quail-----------------------ST*H*QGRY*AAFTQ-SSSTAGTD*SV*CPEGRQ*RWL----FGRR*LSCHNRNDYHNA-YGV-------------------------------
--HRNKSF**EWTRSCCNIPRTR*RWIEPIFRGQSYRYTKTVPQRF?

Quail-----------------------ST*H*QGRY*AAFTQ-SSSTAGTD*SV*CPEGRQ*RWL----FGR**LSCHNRNDYHNA-
YGVS*DSLNP*KTVQDILEFDL*NLT*TQAMVSGRVLM*RQSCKIGSNSLNPT*ASK*KLL--
MRMDEILL*HSQDQVKTD*THF*RSE-------LQIHQNGPAEIL

Quail-----------------------ST*H*QGRY*AAFTQ-SSSTAGTD*SV*CPEGRQ*RWL----FGR**LSCHNRNDYHNA-
YGV*FSCTNGGKTKMLLL*V*L*DTI*QSSKGTIMDILEASPKTYNGVCADPETH*THERRYKIYWNSIFET*HEPRQWYLAEY*CEDSLAKL
AQTA*IQLRHRNKSF**EWTRSCCNIPRTR*RRIEPIFRGQSYRYTKTVPQRF-

Quail-----------------------ST*H*QGRY*AAFTQ-SSSTAGTD*SV*CPEGRQ*RWL----FGRR*LSCHNRNDYHNA-YGVTH---------------------------
---F*RSELQTHQNGPAEIL---------------------------

Turkey-----------------------ST*H*QGRY*AAFTQ-SSSTAGTD*SV*CPEGRQ*RWL----FGRR*LSCHNRNDYHNA-YGV------------------

---Turkey--HRNKSF**EWTRSCCNIPRTR*RWTEPIFRGQSYRHTKTVPQRF?

Turkey -----------------------ST*H*QGRY*AAFTQ-SSSTAGTD*SV*CPEGRQ*RWL----FGRR*LSCHNRNDYHNA-
YGVS*DSLNP*KTVQDILEFDL*NLT*TQALVSGRVLM*RQCCKIGSNSLNPI*ASK*KLL---------------------------------------
MRMDEILL*HSQDQVKMD*THF*RSE-------LQTHQNGPAEIL

Turkey -----------------------ST*H*QGRY*AAFTQ-SSSTAGTD*SV*CPEGRQ*RWL----FGRR*LSCHNRNDYHNA-
YGV*FSCTNGGKTKMLLL*V*L*NTI*QSSKGTIMDILEASPKTYNGVCADPETH*THERRYKIYWNSIFET*HEPRHWYLAEY*CEDSVAKL
AQTA*IQFRHRNKSF**EWTRSCCNIPRTR*RWTEPIFRGQSYRHTKTVPQRF-

Fig. 3. Multiple sequence alignment of the reference amino acids of myostatin gene using muscle from MEGA

Phylogenetic trees represent the evolutionary pathways and a distinction between species trees and gene trees [30]. The most widely used method in constructing phylogenetic tree or dendograms is unweighted pair group method using an arithmetic average (UPGMA). The UPGMA consensus trees on the basis of nucleotide sequences show that, the selected poultry species in this study were closely related. This might be due to the fact that, they belong to members of family Phasianidae. This findings agree with the submission of [31] who observed similar clustering of members of Bovidea family in cattle, sheep and goat. The trees also indicate that, chicken diverge more than other selected poultry species. This might be due to species variability among the selected poultry species. This information may aid in selection of superior animals within and between breeds for genetic improvement of desired traits [23].

5. CONCLUSION

Invitro studies of biologically functional of SNP, is quiet laborious, time consuming and expensive. On the other hand, insilico approach can help us predict the consequences of mutations and explain their affecting role in biological mechanisms. MSTN gene is a potential candidate gene in a QTL detection in livestock species in which mutation effect could be deleterious one, were more than the beneficial ones. The evolutionary pathways shows that, the selected species in this study are member of phasianidae family (chicken, quail and turkey) and more closely related. Positive values of D in Tajima's neutrality test show purifying selection in the selected species. The result obtained in this study could be useful in selection of desirable traits and genetic improvement of livestock species.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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