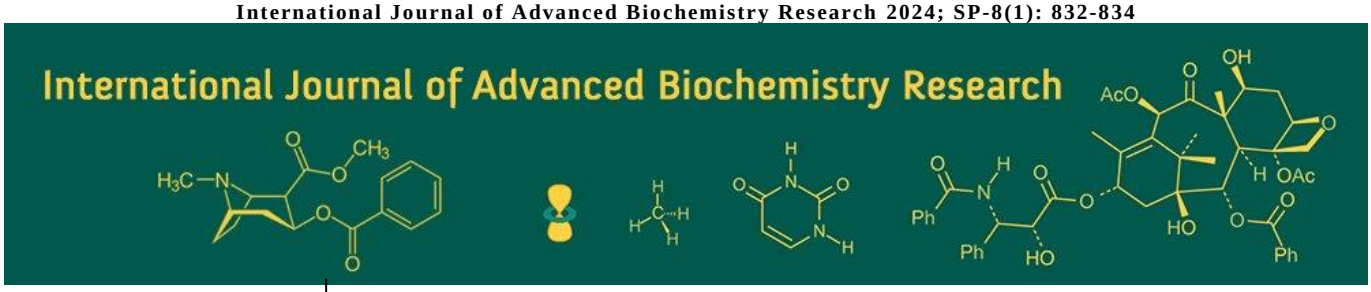

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(1): 832-834
www.biochemjournal.com
Received: 20-11-2023
Accepted: 30-12-2023

Ayushi Singh
Division of Animal Genetics, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh, India

Gaurav Patel
Division of Animal Genetics and Breeding, ICAR-NDRI, Karnal, Haryana, India

Ashok Chaudhary
Division of Animal Genetics, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh, India

Priyanshi Yadav
Division of Veterinary Microbiology, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh, India

Corresponding Author:
Ayushi Singh
Division of Animal Genetics, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh, India

Computational Prediction of deleterious mutation in *ACTN3* gene in cattle

Ayushi Singh, Gaurav Patel, Ashok Chaudhary and Priyanshi Yadav

DOI: https://doi.org/10.33545/26174693.2024.v8.i1Sk.452

Abstract
Actinin Alpha 3 gene (*ACTN3*) has profound role in muscle development and feed efficiency. Therefore, it forms fundamentally important gene in whole mammalian class including beef cattle. Non-synonymous mutations in *ACTN3* gene in human has previously been known to be usually non-tolerant in nature and causes decrease in muscle mass. To extend the knowledge for bovine *ACTN3* gene led to the formulation of in-silico prediction of mutations in the present study. In this study, about 708 mutations were found to be non-synonymous initially and were filtered to eight SNPs using SIFT score. Out of which, one nsSNP (rs723349530, Y392D) was found to have significant deleterious effect as per different scores and algorithms. Protein interaction network revealed connection with proteins having functions on muscle twitching and strength. This is one of its first attempt to characterise non-synonymous mutation of *ACTN3* gene in bovine genome reflecting its importance in muscle growth and hereby, feed efficiency in beef cattle.

Keywords: *ACTN3*, deleterious mutations

1. Introduction
The global human population is projected to reach 9.8 billion by 2050 (FAOSTAT 2018), resulting in a significant increase in demand of high-quality meat and dairy products. Moreover, there is limited land and natural resources for production expansion, which creates a pressing need to develop strategies to improve food production efficiency. Feed provision accounts for up to 75% of total direct costs in beef cattle enterprises, significantly impacting profitability (Nielsen *et al*., 2013) [12]. Feed efficiency is the ratio of feed/forage consumed to animal weight gain over a given time period. Selection of beef cattle for improved feed efficiency has benefited us in two ways: 1) reduced feed intake without compromising growth and product quality (Mao *et al*., 2013) [9], and 2) reduced environmental footprint (Manafiazar *et al*., 2016) [8]. Feed efficiency is a very complex trait affected by numerous genetic and biological mecahnisms (Brunes *et al*., 2021) [3]. The *ACTN3* gene encodes the α-actinin-3 protein (*ACTN3*), which is a component of the contractile apparatus in "fast-twitch" muscle fibres. This protein is responsible for rapid muscle contractions and plays a role in strength and power performance (MacArthur & North, 2007) [7]. *ACTN3* gene has found to have association with different muscle physiology and thereby affects feed efficiency. A recent study on muscle transcriptomes was performed to correlate feed efficiency in Nellore steers as per high or low residual feed intake (Tizioto *et al*., 2016) [15].
Single nucleotide polymorphisms (SNPs) are common and essential variations in the cattle genome, and there is a strong link between variation and certain economically important traits (Rasal *et al*. 2015) [14]. Missense mutations, also known as non-synonymous SNPs (nsSNPs), occur in the coding region and alter the amino acid configuration, potentially affecting the protein's structure and function (Wohlrab, 2006) [16]. The experimental design of mutational changes will be laborious and time-consuming. As a result, it becomes essential and beneficial to perform the fundamental work required for mutation design and protein properties using computational biology (Nailwal and Chauhan, 2017) [10].

Several studies have reported the relationship between SNPs and different diseases and related protein targets (Barreiro *et al*., 2008; Wolf *et al*., 2013) [2, 17]. SNP prediction can be used as a genetic marker to investigate the deleterious effects of SNPs on protein structure and functionality. The goal of this study was to predict the deleterious mutations in *ACTN3* gene of cattle in light of its importance for muscle strength and thereby feed efficiency.

2. Materials and Methods

2.1 Data Retrieval

The data for various SNP variants were retrieved from Ensembl-Biomart Databases (source: dbSNP; http://www.ensembl.org/ biomart/martview/). Variant table was downloaded from Biomart with various information. The associated protein sequence was downloaded from Uniprot (https://www.uniprot.org/uniprotkb/Q0III9).

2.2 Prediction of deleterious nsSNPs

Sorting Intolerant from Tolerant (SIFT) analysis was used to predict the mutation through protein substitution (Ng and Henikoff, 2003) [11]. The deleterious nature of mutation depends on SIFT score which ranges from 0 to1. A score lesser than 0.5 to 0 is considered intolerant mutation (http://sift-dna.org/). SIFT was initially utilized for human studies including cancer. Now, its use has been extended several animal species such cattle, canines (Gharahkhani *et al*., 2011) [6]. Further verification of harmful effect of mutation was performed using software PolyPhen-2 software (Adzhubei *et al*., 2010) [1]. The principle of prediction depends on several factors such as structural and phylogenetic characteristics of amino acid substitution. MutPred2 server was also utilized to further validate probably the deleterious mutation caused in *ACTN3* gene (Pejaver *et al*., 2020) [13].

2.3 Prediction of structural and functional effect of mutation on gene

The structural stability of the mutation on target gene was predicted using MUpro tool. Support Vector Machine algorithm was utilized to predict stability of protein during non-synonymous mutations (Cheng *et al*., 2006) [4]. It predicts stability based on Double Delta G value (Kcal/mol) for the target mutation.

2.4 Analysis of protein network interaction

Protein-protein interaction network was generated using "Search Tool for the Retrieval of Interacting Proteins" (STRING; http://string-db.org/). The interaction network was generated using high confidence scores and other parameters.

3. Results and Discussion

A total of 708 SNPs were filtered out as deleterious in nature in the bovine *ACTN3* gene during variant calling

3.1 Prediction of deleterious mutation using different software

A set of 8 SNP variants were filtered on the basis of SIFT score of 0 with deleterious SIFT class. SIFT predicted a list of mutants with deleterious effect illustrated in Table 1. The polyphen2 software also found that R to C and Y to D are probably damaging having a score of 1 with a specificity of 1 for occurrence of rare alleles identified using GWAS studies. Moreover, these two-point mutations were found to be probably damaging for Mendelian diseases associated with deleterious effect in the target population. A point mutation of E to K and T to I were found to be probably damaging for occurrence of rarer alleles with a score of 0.6. The score for Mendelian diseases was found benign for T to I mutation and thereby, represents relatively less harmful mutation. PolyPhen2 scores are represented in Table 2 for different mutations. Mutation Y to K is found to affect B-factor and relative solvent accessibility with a MutPred2 algorithm score of 0.838. Alteration in transmembrane protein, and ubiquitylation was observed in mutation of E to K. Scores predicted by MutPred2 software are tabulated in Table 3.

Table 1: Deleterious mutation variants predicted by SIFT software

Variant	Location	Point mutation	SIFT class	SIFT score
rs800183570	211	R to C	deleterious	0
rs453306884	392	Y to D	deleterious	0
rs723349530	574	E to K	deleterious	0
rs210333254	741	T to I	deleterious	0

Table 2: Identified damaging mutants using PolyPhen2 software

Mutation	Position	Prediction Score (HumAN div)	HUmaNVAR
R to C	211	1.OO	0.818
E to K	574	0.616	0.748
Y to D	392	1	0.994
T to I	741	0.629	0.409

Table 3: Identified mutants as per MutPred2 algorithm

Point mutation	Position	Known alteration	Score
Y to D	392	Gain of B-factor, Gain of Relative solvent accessibility	0.838
T to I	741	Not identified	0.487
R to C	211	Not identified	0.466
E to K	574	Altered transmembrane protein, Gain of Ubiquitylation	0.714

3.2 Structural and functional effect of *ACTN3* gene

The effect of Mutant SNP variants on *ACTN3* gene stability was assessed through MuPro software. Prediction of structural stability of SNP is predicted in Table 4 by MuPro software. A protein-protein interaction network was constructed using STRING browser to better predict the functionality of *ACTN3* gene with high confidence interval (<= 0.7) The gene was found to be functionally related to *MYOZ1*, *MYBPC2*, *ACTN2*, *TNNI1*, *TMOD4*, *TNNT3*, *MYL1*, *TNNC2*, *MYL1*, *MYH1*.STRINGnetwork is illustrated in Figure 1.

Table 4: Structural stability and Gibbs free energy of Mutant SNP variants

SNPs	DDG value	Prediction
rs800183570	-0.91581953	decrease
rs453306884	-0.82698141	decrease
rs723349530	-0.47038116	decrease
rs210333254	-0.40348408	decrease

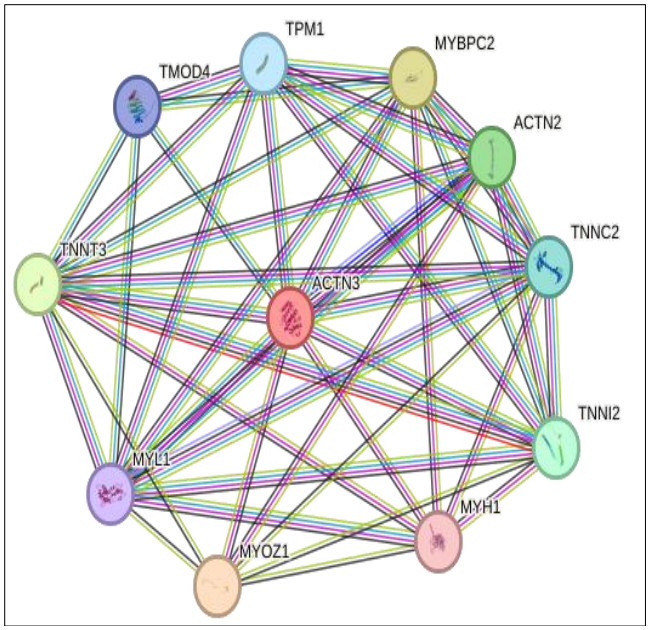

Fig 1: Protein-protein interaction network using STRING API

4. Conclusion

Beef meat consumed world-wide and forms an important pillar of food security. However, improvement in productivity of beef depends on increasing feed efficiency in cattle. *ACTN3* gene is crucial for development of muscle mass and plays a significant role in feed efficiency. However, deleterious mutations can affect functioning of the gene and ultimately change the protein structure. The current study identified and predicted eight deleterious nsSNPs in the bovine *ACTN3* gene through computational approach. Several structural and protein interactions were revealed and known to affect muscle function. This study suggests that Y392D mutation for rs723349530 SNP variant has significant deleterious effect and can be further investigated for its effect on gene functioning through *in vivo* studies.

5. Conflict of interest

Authors declare that they have no conflict of interest in the present study.

6. References

1. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, *et al*. A method and server for predicting damaging missense mutations. Nature methods. 2010 Apr;7(4):248-9.
2. Barreiro LB, Laval G, Quach H, Patin E, Quintana-Murci L. Natural selection has driven population differentiation in modern humans. Nature genetics. 2008 Mar;40(3):340-5.
3. Brunes LC, Baldi F, Lopes FB, Lôbo RB, Espigolan R, Costa MF, *et al*. Weighted single-step genome-wide association study and pathway analyses for feed efficiency traits in Nellore cattle. Journal of Animal Breeding and Genetics. 2021 Jan;138(1):23-44.
4. Cheng J, Randall A, Baldi P. Prediction of protein stability changes for single-site mutations using support vector machines. Proteins: Structure, Function, and Bioinformatics. 2006 Mar 1;62(4):1125-32.
5. FAO F. Food and agriculture organization of the United Nations. Rome, URL: http://faostat.fao.org.2018 Jul 25.
6. Gharahkhani P, O'Leary CA, Kyaw-Tanner M, Sturm RA, Duffy DL. A non-synonymous mutation in the canine Pkd1 gene is associated with autosomal dominant polycystic kidney disease in Bull Terriers. PLoS One. 2011 Jul 27;6(7):e22455.
7. MacArthur DG, North KN. *ACTN3*: A genetic influence on muscle function and athletic performance. Exercise and sport sciences reviews. 2007 Jan 1;35(1):30-4.
8. Manafiazar G, Zimmerman S, Basarab JA. Repeatability and variability of short-term spot measurement of methane and carbon dioxide emissions from beef cattle using Green Feed emissions monitoring system. Canadian Journal of Animal Science. 2016 Aug 4;97(1):118-26.
9. Mao F, Chen L, Vinsky M, Okine E, Wang Z, Basarab J, *et al*. Phenotypic and genetic relationships of feed efficiency with growth performance, ultrasound, and carcass merit traits in Angus and Charolais steers. Journal of Animal Science. 2013 May 1;91(5):2067-76.
10. Nailwal M, Chauhan JB. Analysis of consequences of non-synonymous SNPs of USP9Y gene in human using bioinformatics tools. Meta Gene. 2017 Jun 1;12:13-7.
11. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic acids research. 2003 Jul 1;31(13):3812–3814.
12. Nielsen MK, MacNeil MD, Dekkers JC, Crews Jr DH, Rathje TA, Enns RM, *et al*. Life-cycle, total-industry genetic improvement of feed efficiency in beef cattle: Blueprint for the Beef Improvement Federation. The Professional Animal Scientist. 2013 Dec 1;29(6):559-65.
13. Pejaver V, Urresti J, Lugo-Martinez J, Pagel KA, Lin GN, Nam HJ, *et al*. Inferring the molecular and phenotypic impact of amino acid variants with MutPred2. Nature communications. 2020 Nov 20;11(1):5918.
14. Rasal KD, Shah TM, Vaidya M, Jakhesara SJ, Joshi CG. Analysis of consequences of non-synonymous SNP in feed conversion ratio associated TGF-β receptor type 3 gene in chicken. Meta Gene. 2015 Jun 1;4:107-117.
15. Tizioto PC, Coutinho LL, Oliveira PS, Cesar AS, Diniz WJ, Lima AO, *et al*. Gene expression differences in Longissimus muscle of Nelore steers genetically divergent for residual feed intake. Scientific reports. 2016 Dec 22;6(1):39493.
16. Wohlrab H. The human mitochondrial transport/carrier protein family. Nonsynonymous single nucleotide polymorphisms (nsSNPs) and mutations that lead to human diseases. Biochimica et Biophysica Acta (BBA)-Bioenergetics. 2006 Sep 1;1757(9-10):1263-70.
17. Wolf AB, Caselli RJ, Reiman EM, Valla J. APOE and neuroenergetics: an emerging paradigm in Alzheimer's disease. Neurobiology of aging. 2013 Apr 1;34(4):1007-17.