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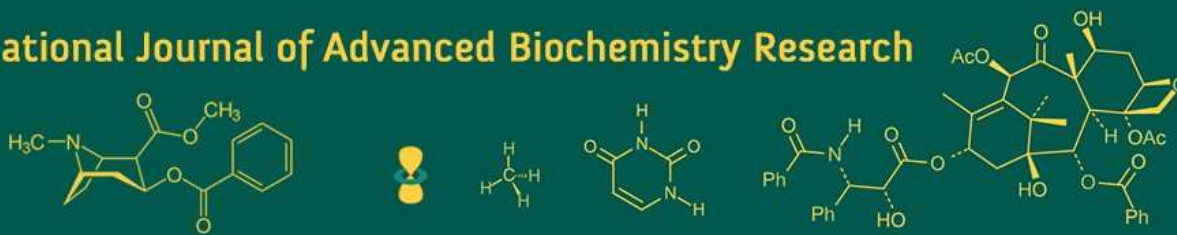
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In-silico detection of deleterious mutation in LEPR gene in dairy cattle

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Abstract
LEPR gene specific deleterious mutations were identified in dairy cattle using *in silico* approach. SNP variants associated gene were retrieved and associated protein sequence was accessed from UniProt database. SNP variants were filtered for Sorting Intolerant from Tolerant (SIFT) algorithm with a threshold of $<= 0.5$. A total of 238 SNPs were found to be deleterious point mutations. These missense SNPs were further filtered and ordered as per SIFT score. Only four SNPs with lower SIFT score were considered highly missense mutations. These SNPs with point mutations at F88Y, S461G, T941M, L912R were further analysed using PolyPhen-2 and MutPred2 algorithm. Further, structural stability was checked through calculation of Gibbs Free energy using MUpro software. Interactions OF LEPR gene with other proteins and genes were identified through STRING network. STRING interactions revealed its association with LEP, JAK1, JAK2, JAK3, NPY, POMC, INS, AGRP, SOCS3 and IL6 gene. These genes were affecting processes such as feed intake, immunity, energy homeostasis, insulin signalling in cattle. Overall, this is one of its first attempt to predict deleterious mutations in LEPR gene using *in silico* approach. It may be useful for preliminary identification of missense mutations in LEPR gene in cattle.

Keywords: Non-synonymous, *in silico*, LEPR, mutations

1. Introduction

Livestock products form one of the major sources of energy and good quality animal protein for ever rising human population. According to FAOSTAT (2018) [24], there will be a population of 9.8 billion by 2050. With the rampant increase in human population, there will be increased demand of animal protein and food as a whole. According to the modelling, there will be a 21% increase in global meat consumption per person and a 63% increase worldwide by 2050 (Revell, 2015) [6]. It drives a need for innovative and sustainable solutions to meet out increased demands of animal protein in future.

Cattle is major reared livestock species in the world. It is reared for milk and beef production. There is large amount of cattle milk consumption around the world varying around 10–212 kg per person per year (WHO report, 2018) [7]. Even, the beef consumption around the world in 2019 had a whopping share of about 11 percent (OECD meat consumption report, 2024) [8]. Additionally, protein availability from beef is likely to grow up to 8% by the year 2031. Current scenario suggests for innovative solutions to uptake the challenge for increasing milk and meat production to meet out demands of future. The milk production and meat production are complex traits affected by several factors such as feed, management and genetic predisposition of animal. Genetic factors play an important role in augmenting and enhancing milk and meat production around the world. Several genes affect production traits such as DGAT1 (Samuel *et al*., 2022) [12], LEP (Hernandez *et al*., 2016) [25], LEPR (da Silva *et al*., 2012) [11]. Leptin is an important protein secreted by adipose tissue (Perez-Montarelo *et al*., 2013) [26] and its expression is mediated by Leptin receptor (LEPR) present in the hypothalamus. The leptin receptor (LEPR) gene is a crucial gene which affects various physiological processes particularly, feed intake, metabolism, energy balance, and reproduction of cattle (Raza *et al*., 2020) [9]. Moreover, the leptin system affects the reproduction and fertility in cows especially in case of repeat breeders (Ninpetch *et al*., 2022) [1].

It also influences endocrine signalling in male and female reproductive system (Williams *et al*., 2002) [2]. Studies have shown that leptin plays a physiological role in cattle by controlling food intake through inhibiting hunger and influencing feed efficiency. Animals with higher circulating leptin levels tend to be more feed efficient and exhibit lower residual feed intake (Mota *et al*., 2017) [4].

Single nucleotide polymorphisms (SNPs) are important variations found in the cattle genome, and are strongly found to affect economically important traits (Rasal *et al*. 2015) [13]. Several Leptin and its receptor gene polymorphism studies have been carried out to study the variation of different productivity taris across groups (Raza *et al*., 2020) [9]. A polymorphism study in exon 2 of LEPR gene in Madura cattle has been found to be associated with body weight (Kuswati *et al*., 2022) [5]. However, few SNP variations and point mutations are deleterious in nature. Previous studies in humans have identified negative effect of deleterious mutations in LEPR gene on humans leading to increased incidence of obesity (Huvenne *et al*., 2015) [14]. The deleterious mutations in LEPR gene can also negatively affect the production and reproduction traits of cattle. Several in-silico deleterious mutation prediction studies have been carried out for several genes. However, there is no such studies attempted for Leptin receptor gene (LEPR). Therefore, the present study is carried out with an attempt to predict in-silico non-synonymous mutations in LEPR gene in cattle using different prediction algorithms.

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 LEPR gene protein sequence was downloaded from Uniprot (https://www.uniprot.org/uniprotkb/A0A3Q1MPM8).

2.2 Prediction of deleterious nsSNPs
Sorting Intolerant from Tolerant (SIFT) analysis was used for detection of non-synonmous mutation through protein substitution (Ng and Henikoff, 2003) [15]. 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 (Ali *et al*., 2022) [16], canines (Gharahkhani *et al*., 2011) [17]. Further verification of harmful effect of mutation was performed using software PolyPhen-2 software (Adzhubei *et al*., 2010) [20]. 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 LEPR gene (Pejaver *et al*., 2020) [19].

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) [18]. 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. (Szklarczyk *et al*., 2023) [21].

3. Results & Discussion
A total of 238 SNPs were categorized as deleterious in nature in the bovine LEPR gene during variant calling.

3.1 Prediction of deleterious mutation using different software
A set of 137 SNP variants were found to be have a SIFT score of 0 with deleterious SIFT class. Out of which, SNPs with SIFT score of 0, 0.02, 0.03 were filtered and used for further mutation analysis. Table 1 shows the anticipated list of mutations with deleterious effects. Additionally, prediction using polyphen2 software found that S to G and L to R point mutation are probably damaging having a score of 1 with a specificity of 1 for occurrence of rare alleles identified using GWAS studies. A point mutation from T to M was predicted to be possibly damaging with a score of 0.953. However, point mutation from F to Y was found to be benign in nature. The possibly damaging mutations were previously reported in Mendelian diseases causing deleterious effect in the target population. Table 2 shows the PolyPhen2 scores for the various point mutations.

Table 1: Deleterious mutation variants predicted by SIFT software in cattle

Variant	Location	Point mutation	SIFT class	SIFT score
rs477721419	88	F to Y	deleterious	0
rs211411897	461	S to G	deleterious	0.02
rs133672995	945	T to M	deleterious	0
rs471482820	912	L to R	deleterious	0

Table 2: PolyPhen-2 prediction score for various point mutations

Mutation	Position	Prediction Score (Human div)	Human VAR Prediction score
F to Y	88	0.029	0.93
S to G	461	1.00	0.983
T to M	945	0.953	0.586
L to R	912	1.00	1.00

Table 3: Predicted mutations by MutPred2 algorithm

Point mutation	Position	Score
F to Y	88	0.414
S to G	461	0.273
T to M	945	0.164
L to R	912	0.584

3.2 Structural and functional effect of LEPR gene
The effect of Mutant SNP variants on LEPR 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 LEPR gene with high confidence interval (<= 0.4) The gene was found to be functionally related to LEP, JAK1, JAK2, JAK3, NPY, POMC, INS, AGRP,

SOCS3 and IL6 genes. Pro-opiomelanocortin (POMC) regulates the energy balance and ingestion behaviour in animal (Zhang *et al*., 2009) [22]. Insulin (*INS*) gene helps in insulin signalling and dietary restriction in cattle. (AGRP) is an important modulator of nutrient partitioning and energy homeostasis through hypothalamus regulation (Richards and Proszkowiec-Weglarz., 2007) [23]. STRING network is illustrated in Figure 1.

Table 4: Gibbs Free energy and prediction of structural stability by MUpro software

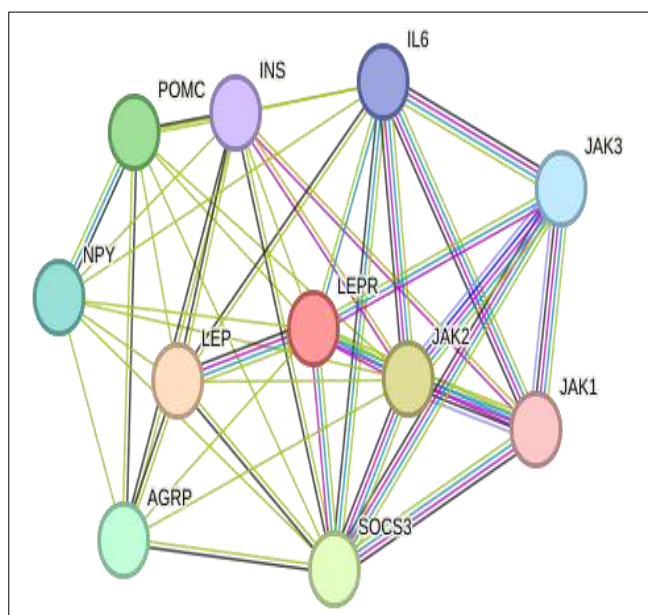| SNPs	DDG value	Prediction
rs477721419	-1.3038333	decrease
rs211411897	-1.2205093	decrease
rs133672995	-0.63164138	decrease
rs471482820	-1.5645831	decrease

Fig 1: STRING network interactions of LEPR gene

4. Conclusion
LEPR gene is well known as productive trait marker for dairy cattle. It is crucial for several production and reproduction traits of cattle. SNP polymorphism leads to different variants of LEPR gene which can be either beneficial or deleterious in nature. Deleterious mutations can negatively affect the overall physiology of dairy cattle. The current study predicted about 238 missense mutations. With the use of different algorithm for mutation prediction, mutation of L(Leucine) to R (Arginine) at 912 position was found to be highly deleterious. Additionally, STRING interactions revealed genes affecting immunity, energy homeostasis, insulin signalling and nutrient partitioning.

5. Ethical approval
Not applicable. No procedures requiring ethics approval were performed in this study.

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

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