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Research Article

Identification of deleterious SNPs in bovine *HPRT* gene by *in silico* approach

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Abstract

Hypoxanthine phosphoribosyltransferase is a key player in the purine salvage pathway; hence its transcribing *HPRT1* gene is treated as an important housekeeping gene in the mammalian genome. Non-synonymous (ns) changes in the *HPRT1* gene are generally not tolerated and may cause genetic diseases including Lesch-Nyhan Syndrome (LNS) in humans. To find out such genetic disease in bovines due to *HPRT1* gene (on X-chromosome) mutation, this study was carried out. In this study, the effects of nsSNPs in the *HPRT1* gene of cattle retrieved from the Ensembl-Biomart were assessed *in silico*. These nsSNPs were analysed for deleterious effects as well as structural changes associated with the mutants. Among 22 nsSNPs, a total of nine were predicted as deleterious by SIFT, PROVEAN and PANTHER online tools. All nine SNPs showed a decrease in stability using I-mutant 2.0 and MuPro. One nsSNP (rs465703426, G140V) with the highest deleteriousness based on scores of these tools was found to be the most damaging to the native structure along with significant deviation in energy minimization. The Protein network analysis revealed the linkage of 10 other proteins associated with important functions like cell growth and nucleotide biosynthesis. Alteration in *HPRT1* may lead to affection to these linked protein functions and might affect the regulation of cellular growth. In this first attempt to assess the deleterious effects of mutations in the bovine *HPRT1* gene, the study provides an indication of its harmful implications in the purine salvage pathway.

Keywords: Bovine, *HPRT1* gene, *In silico* analysis, Non-synonymous, SNP

Highlights

- Effects of nsSNPs in the *HPRT1* gene of cattle were assessed *in silico*.
- A total of nine nsSNPs were predicted as deleterious by SIFT, PROVEAN and PANTHER online tools.
- NsSNP rs465703426 (G140V) was found to be the highest deleterious based on scores (of *in silico* tools).
- The Protein network of *HPRT1* revealed the linkage of 10 other proteins associated with cell growth and nucleotide biosynthesis.

INTRODUCTION

To understand the genetic basis of genetic diseases, Single-nucleotide polymorphisms (SNPs) prove very much important at the DNA level. Non-synonymous SNPs (nsSNPs) cause changes in the residues of amino acids and contribute significantly to the functional diversity of encoded proteins (Yates *et al.,* 2013). Non-synonymous SNPs influence gene regulation through the modification of DNA and transcriptional binding and also influence protein functionality in visual, hormonal, and other stimulants involved in signal transduction (Rajasekaran *et al*., 2007; Gfeller *et al*., 2014).

Over the last few years, computing algorithm advancements were found effective to find out deleterious nsSNPs in candidate genes and the impact of such mutation on protein structure and function. As it is very tiresome, time-consuming and costly to find mutational changes through experimental design, presently, *in Silico* tools like SIFT, PROVEAN, PANTHER and I-Mutant are used for identifying deleterious nsSNPs in the coding regions. A number of studies have been conducted to detect harmful nsSNPs by using these tools for genes such as *RASSF5*, *GalNAc-T1*, *MECP2*, *BRAF*, *TNF-α*, *BARD-1* and *IGF1R* in humans (Hossain *et al.*, 2020; Ali Mohamoud *et al.*, 2020; Desai and Chauhan, 2016; Hussain *et al.*, 2012; Dabhi and Mistry, 2014; Alshatwi *et al.*, 2012; De Alencar and Lopes, 2010). Similarly, in the bovine such study has been conducted in the *SLC11A2* gene (Patel *et al.*, 2015) and *CSN3* gene (Patel and Chauhan, 2018); deleterious SNPs were predicted using SIFT, PolyPhen and Panther.

Hypoxanthine phosphoribosyl transferase (HPRT) is an integral element of the purine phosphoribosyl-transferases (PRT) and plays a central role in the

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generation of purine nucleotides through the purine salvage pathway. It catalyses the development of nucleotide monophosphates using preformed purine and phosphoribosyl pyrophosphate (PRPP) substrates (Craig and Eakin, 2000). In humans, the *HPRT1* gene shows classical X-linked inheritance, and the lethal mutation in the gene can lead to serious human neurological and metabolic disorders, Lesch-Nyhan Syndrome due to hypoxanthine-guanine phosphoribosyltransferase enzyme deficiency (Duan *et al.*, 2004; Fu *et al.*, 2014; Harris, 2018). The *HPRT1* gene located on the X chromosome produces purine nucleotides, and mutation in this gene leads to abnormal expression of the neurotransmitter dopamine and neuron function (Xia, 2013).

In this study, the information on SNPs of the *HPRT1* gene in cattle was retrieved from the Ensembl-Biomart and was further assessed for their functional effects using online tools. The deleterious effects of nsSNPs were further confirmed *in silico* by predicting protein stability, its structural and functional analysis, and its effect on the protein network. The study predicted rs465703426 as the highest deleterious nsSNP (G140V) with a significant damaging effect on the native structure, which further affects important functions like cell growth and nucleotide biosynthesis. This study is the first attempt to assess the deleterious effects of mutations in the bovine *HPRT1* gene and may be helpful to provide the lead for further *in vivo* studies on cellular and physiological aspects.

MATERIALS AND METHODS

Identification of deleterious nsSNPs: The data of the *HPRT1* gene were retrieved from Ensembl-Biomart Databases (source: dbSNP; http://www.ensembl.org/biomart/martview/) and Uniprot (https://www.uniprot.org/uniprot/Q3SZ18/). We retrieved the information of SNPs (SNP ID, location, Gene stable ID, residue alteration, etc.). We filtered all 723 SNPs for screening.

Sorting Intolerant from Tolerant (SIFT) analysis was performed for allele substitution at various positions for the given query sequences (Ng and Henikoff, 2003). The SIFT predicted the deleteriousness of the SNP in the form of a tolerance index (TI) score ranging from 0.0 to 1.0, with a TI score of 0.05 or less as intolerant or deleterious (Ng and Henikoff, 2001; 2003; 2006). Further, the obtained result was determined by the Codon's median conservation sequence (MSCS). The deleterious SNP with MSCS ≥ 3.25 value predictions revealed low confidence.

Further, to verify the identified deleterious SNPs from SIFT, the protein analysis through evolutionary relationships (PANTHER) tool was used to analyse the evolutionary relationship and interaction of protein using PSEP (position-specific evolutionary preservation) score (Tang and Thomas, 2016). Based on the confidence score, predictions were classified as "probably damaging" (affect the protein structure), "possibly damaging" (may affect the protein structure) and "benign" (no phenotypic effects). Protein Variation Effect Analyser (PROVEAN) web server tool was used to predict the biological functional effect on a protein due to amino acid substitutions (Choi *et al*., 2012). On the basis of a cut-off value of -2.5 for well-adjusted accuracy, PROVEAN predictions were made as deleterious ($\leq$-2.5) or Neutral (>-2.5). The protein sequence of the *HPRT1* gene from UniProt was provided as input in PANTHER and PROVEAN server in FASTA format with altered protein variation and position for the functional impact prediction.

Prediction of structural and functional effect on bovine HPRT1 protein: Support vector machine (SVM) based I-Mutant2.0 and MUpro tools were used for the prediction of protein stability due to changes in non-synonymous mutations in proteins. Prediction of stability was given on the basis of the Double Delta G value (Kcal/mol) for the given mutation (Capriotti *et al*., 2005; Cheng *et al*., 2006). HOPE version 1.1.1 (https://www3.cmbi.umcn.nl/hope/), an online web server, was used to recognise the structural effect of non-synonymous change in the HPRT1 protein sequence. It also provided 3D structure visualization of altered protein and superimposition of wild and mutant after providing altered protein sequence as input (Venselaar *et al.*, 2010). For functional analysis of the HPRT1 sequence, both native and mutant protein sequences were submitted to SWISS-MODEL. The predicted models were retrieved in PDB format, which were visualized with their bonds using PyMOL (https://pymol.org/). YASARA Minimization (http://www.yasara.org/minimizationserver.htm) web server was used to minimize energy in protein structures (Krieger *et al.,* 2009). Further, the YASARA View program (http://www.yasara.org/viewdl/) was used for visualization. Non-bonded atomic interactions among different types of atoms were assessed by using the ERRAT algorithm (Colovos and Yeates, 1993).

Analysis of protein network interaction: "Search Tool for the Retrieval of Interacting Proteins" (STRING; http://string-db.org/), a freely available web-based tool, was used for visualization of the bovine HPRT1 protein interaction network (Szklarczyk *et al.*, 2010).

RESULTS

A total of 723 SNPs including 22 (3.0%) non-synonymous and 14 (1.9%) synonymous were filtered out in the bovine *HPRT1* gene during variant calling. The highest number of SNPs were present in intronic region (475; 65.7%), followed by the downstream region (76; 10.51%), upstream region (67; 9.27%) and UTR (54; 7.5%) region. Two indels were frameshift variants, and five SNPs as stop gain in the *HPRT1* gene.

Identification of deleterious nsSNPs in bovine HPRT1: Initially 13 deleterious out of 22 non-synonymous SNPs in the bovine *HPRT1* gene were identified based on SIFT score (Table 1). Analysis of 22 nsSNPs for functional impact on protein carried out by PANTHER-PSEP revealed 19 as damaging. All nsSNPs were further analysed by PROVEAN for deleterious effects on protein function, which identified 13 nsSNPs as deleterious based on Tolerance Index (TI) score. Among these nsSNPs, only 9 SNPs (Fig. 1) (D31N, D31G, V35A, K103E, G140V, L146S, V189A, A192G, N196K) were found to be common deleterious in SIFT, PROVEAN and PANTHER 3 tools (Table 1). The G140V SNP (rs465703426) was found to be the most deleterious, based on scores of SIFT, PANTHER and PROVEAN.

Structural and functional effect on bovine HPRT1 protein: On analysis of the impact of these nsSNPs on the stability on HPRT1 protein using I-Mutant 2.0 and MUpro, all nine common deleterious nsSNP, including G140V showed decrease in protein stability based on DDG value at pH 7 and temp 25°C (Table 2).

For analysis of G140V SNP, Project HOPE predicted it as probably damaging to the HPRT1 protein structure, as the valine residue in mutant was found to be larger and more hydrophobic in comparison to glycine of wild allele at 140 amino acid position. Ribbon-presentation by Project HOPE inferred that glycine, as a most flexible residue and highly conserved, might be required for protein's function. So, the local backbone will be forced into an erroneous conformation if a mutation occurs at this position into another residue like valine. Therefore, mutation at the conserved residue may lead to damaging the protein structure (Fig. 2).

PyMOL predicted H-bonds in native (glycine) and mutant (valine) residues at 140 position of HPRT1 protein models obtained by SWISS-MODEL (Fig. 3). Total energy of native structure was found to be -368953.3 kJ/mol (Z score: -1.92) prior to the energy minimisation stage and -553655.1 kJ/mol (Z score: -0.28) after energy reduction using YASARA. The overall energy increase of mutant HPRT1 before (-354833.5 kJ/mol, Z score:-2.08) and after (-488935.1 kJ/mol, Z score: -0.38) energy minimization, exhibiting larger deviation in mutant protein. Z-score of mutant protein was also found to be lower than that of wild, further suggesting the native structure to be better in comparison to the mutant. Overall quality assessed by ERRAT was also found to be better in native (95.062) than mutant structure (94.938). In the protein-protein interaction analysis by STRING, a total of ten proteins were found to be associated with functional HPRT1 protein with a high confidence score (≤ 0.95) (Fig. 4).

Table 1. Predicted deleterious nsSNPs on *HPRT1* gene using SIFT, PROVEAN and PANTHER-PSEP

Sl. N0.	SNPs	Chromosomal position	Mutation	SIFT		PROVEAN		PANTHER	
				Score	Prediction	Score	Prediction	Score	Prediction
1	rs480078398	18185040	L8F	0.09	tolerated	-	-	-	-
2	rs467147199	18186401	F88Y	0	deleterious	-2.375	Neutral	1500	PD
3	rs455581885*	18186427	D31N	0.01	deleterious	-4.241	Deleterious	1237	PD
4	rs474270939*	18186428	D31G	0.01	deleterious	-5.963	Deleterious	1237	PD
5	rs456606672	18186438	K34N	0.3	tolerated	-1	Neutral	361	PD
6	rs475631992*	18186440	V35A	0.02	deleterious	-3.307	Deleterious	842	PD
7	rs458030519	18186463	M43L	0.52	tolerated	-0.803	Neutral	750	PD
8	rs438022691	18187996	E56G	0.05	tolerated	-3.257	Deleterious	324	PD
9	rs456610898	18187998	M57V	0.25	tolerated	-0.692	Neutral	750	PD
10	rs110835586	18188019	A64T	0.05	deleterious	-2.058	Neutral	842	PD
11	rs380695996	18188118	V97L	0.09	tolerated	-2.653	Deleterious	750	PD
12	rs440355678*	18188136	K103E	0	deleterious	-3.543	Deleterious	1368	PD

Cont. Table 1.

Table 1., Cont. ...

Sl. N0.	SNPs	Chromosomal position	Mutation	SIFT		PROVEAN		PANTHER	
				Score	Prediction	Score	Prediction	Score	Prediction
13	rs465703426*	18203518	G140V	0	deleterious	-8.148	Deleterious	4200	PD
14	rs436025328	18203530	Q144P	0.04	deleterious	-2.614	Deleterious	176	PB
15	rs111004439*	18203536	L146S	0	deleterious	-5.616	Deleterious	4200	PD
16	rs469901823	18203561	K154N	0.74	tolerated	-0.215	Neutral	1	PB
17	rs463341786*	18207296	V189A	0	deleterious	-3.364	Deleterious	1500	PD
18	rs445751083*	18207305	A192G	0.02	deleterious	-3.163	Deleterious	1237	PD
19	rs464299812*	18207318	N196K	0	deleterious	-5.14	Deleterious	1500	PD
20	rs434929541	18207338	N203S	0.11	tolerated	-3.103	Deleterious	842	PD
21	rs451690597	18207878	S209R	0.03	deleterious	-1.794	Neutral	750	PD
22	rs474837192	18207904	A218V	0.82	tolerated	0.829	Neutral	361	PD

*Predicted deleterious nsSNPs by all three tools; PD> Probably damaging; PB> Probably benign

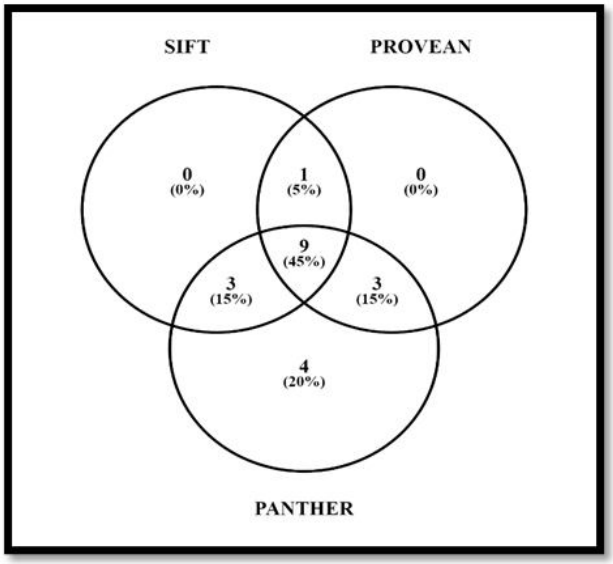
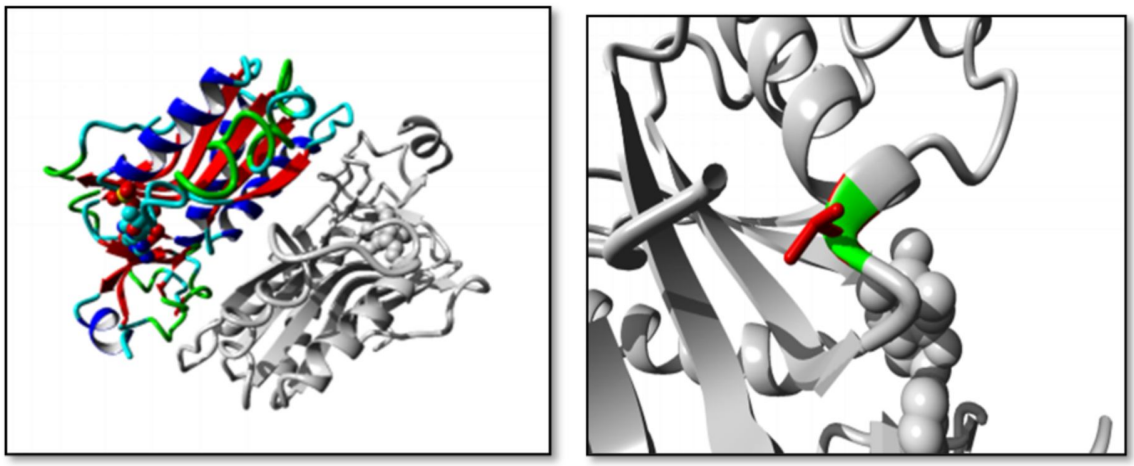


Fig. 1. Venn diagram for the comparison of numbers of predictions made by SIFT, PANTHER and PROVEAN

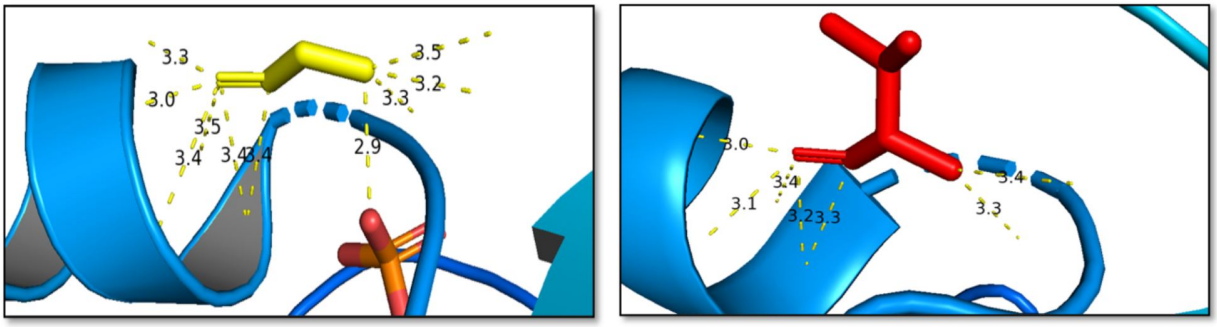
Table 2. Prediction of deleterious nsSNPs on protein stability using I-Mutant 2.0 and MuPro

Sl. No.	SNPs	Mutation	I-Mutant		MuPro	
			DDG value	prediction	DDG value	prediction
1	rs455581885	D31N	0.13	Decrease	-0.72489439	Decrease
2	rs474270939	D31G	-0.5	Decrease	-1.4626318	Decrease
3	rs475631992	V35A	-1.4	Decrease	-1.6331983	Decrease
4	rs440355678	K103E	-1.07	Decrease	-0.19646097	Decrease
5	rs465703426	G140V	-0.76	Decrease	-0.81747959	Decrease
6	rs111004439	L146S	-1.82	Decrease	-1.7404247	Decrease
7	rs463341786	V189A	-2.16	Decrease	-0.66709602	Decrease
8	rs445751083	A192G	-1.44	Decrease	-1.0000462	Decrease
9	rs464299812	N196K	-1.76	Decrease	-1.1931972	Decrease



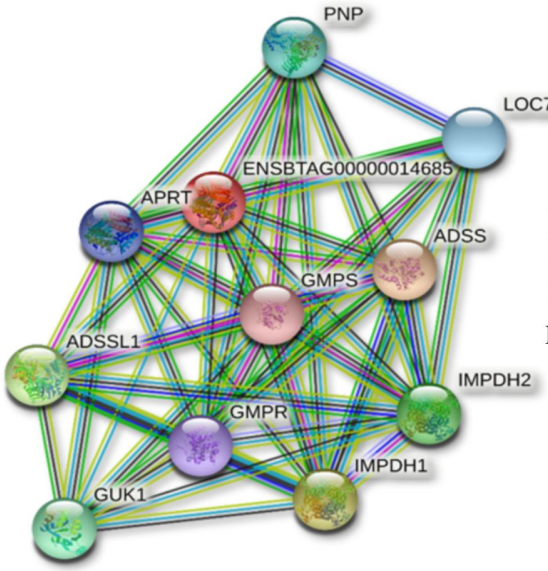
(a) (b)

Fig. 2(a). Predicted tertiary structure (Ribbon-presentation) of mutant HPRT1 protein (G140V) using Project HOPE (Alpha helix- blue, beta strand- red, turns- green, 3/10 helix- yellow, random coiling- cyan, other molecules are in grey colour) (b) Substitution of glycine (green, wild) by valine residue (red, mutant) at 140 residue position of bovine HPRT1 protein



(a) (b)

Fig. 3. Molecular visualization of residue at 140 position with H-bonds (distance in Å) in HPRT1 protein using PyMOL (a) Glycine (yellow) (b)Valine (red)



[HPRT1 protein is shown as ENSBTAG00000014685; colour codes for edges are- sky blue: from curated databases, pink: experimentally determined, green: gene neighbourhood, red: gene fusions, blue: gene co-occurrence, yellow: text mining, black: co-expression and purple: protein homology]

Fig. 4. Protein interaction network of HPRT1 using STRING

DISCUSSION

The Hypoxanthine-guanine phosphori bosyltransferase (HGPRT) plays a central role in the generation of purine through the nucleotide salvage pathway. In human, the *HPRT1* gene shows classical X-linked inheritance, and the lethal mutation in gene can lead to serious human neurological and metabolic disorders, Lesch-Nyhan syndrome due to hypoxanthine-guanine phosphoribosyltransferase enzyme deficiency (Duan *et al*., 2004; Fu *et al*., 2014; Harris, 2018). In the present study, we tried to identify such deleterious mutations in the bovine *HPRT1* gene, using *in silico* approach.

Among 723 SNPs of the bovine *HPRT1* gene retrieved from Ensembl-Biomart, a total of 22 SNPs were screened as non-synonymous. Those SNPs were further used for the identification of deleteriousness using three online tools. SIFT identified 13 nsSNPs as deleterious on the basis of tolerance index (Ng and Henikoff, 2001; Ng and Henikoff, 2003; Ng and Henikoff, 2006); however, only 9 SNPs (D31N, D31G, V35A, K103E, G140V, L146S, V189A, A192G, N196K) were further verified using PANTHER and PROVEAN tools. Generally, the tools use the threshold values for each of the SNP based on the sequence conservations.

A number of such studies have been conducted to detect harmful SNPs by examining potential genes - *RASSF5*, *MECP2*, *CSN3*, *BRAF*, *TNF-α*, *BARD-1*, *IGF1R* and *SLC11A2* in human and animals (Hossain *et al*., 2020; Desai and Chauhan, 2016; Patel and Chauhan, 2018; Hussain *et al*., 2012; Dabhi and Mistry, 2014; Alshatwi *et al*., 2012; De Alencar and Lopes, 2010; Patel *et al*., 2015). In the bovine *SLC11A2* gene, deleterious SNPs were predicted using SIFT, PolyPhen and Panther (Patel *et al*., 2015). Similarly, deleterious SNPs were also reported in the bovine *CSN3* gene (Patel and Chauhan, 2018). In this study, three tools were used to predict the deleterious mutations more accurately and to overcome the limitations of each of the tools. The SIFT provides about 30% as false negative and 20% as false positive results (Ng and Henikoff, 2003; 2006). However, it was more accurate than formerly used BLOSUM62 mainly because of including the information on the protein of interest (Ng and Henikoff, 2006). Since the features used by the algorithms differ in such prediction tools, diverse result outputs of these tools are always expected. In a comparison of these tools on non-human protein variant datasets, the sensitivity was found to be higher in SIFT, whereas the specificity in PROVEAN. Accuracy was found to be almost the same for both tools (Choi *et al*., 2012).

The stability of protein is very much important from the aspect of its structural and functional activities (Deller *et al*., 2016). Every change in protein stability could lead to misfolding, degradation or aberrant protein conglomeration (Witham *et al*., 2011). I-mutant and MuPro, two widely used tools, showed a decrease in stability. The nsSNP G140V, with the maximum score, also revealed the structural aberration of protein and seemed to be the highest deleterious. Project HOPE-predicted it as probably damaging to the HPRT1 protein structure due to mutation of glycine (wild) to valine (mutant) at mutation site (140 amino acid position). The torsion angle for that residue was unusual, as glycine, the most flexible among all residues, can make the angles much more easily, and that could have been difficult for valine. So, the local backbone will be forced into an erroneous conformation if a mutation occurs at this position into another residue. Also, the wild-type residue was found with a high level of conservation. In similar work, Hossain *et al*. (2020) used I-mutant and Project HOPE for analysing human *RASSF5* gene mutations and found that there was reduced stability in mutant protein and declined protein binding affinity, leading to disassociation of several signal cascades. Such protein model analysis using I-Mutant and HOPE was also carried out for the bovine *CSN3* gene (Patel and Chauhan, 2018) and for the human *TNF-α* gene (Dabhi and Mistry, 2014), which revealed functional deviations of mutant protein, to some extent, due to decreased protein stability.

Protein modelling proved to be an effective *in silico* technique for testing the impact of these nsSNPs on protein structural stability. Comparison of total energy between native and model structure showed deviation in the mutant model G140V, as higher total energy, which made the model unstable in comparison to native. As seen, the number of polar contacts varied as some amino acids changed, affecting the overall energy of the protein and implying a loss in protein stability. The Z-score of the mutant model was found with a lower score in comparison to the native, further suggesting the native structure to be better in comparison to the mutant. Analysis through ERRAT also suggested better quality of native structure over the mutant *HPRT1* in bovines.

To analyse the downstream effect of the mutated *HPRT1*, we further predicted the interaction of HPRT protein with other proteins involved in purine nucleotide bio-synthesis and regulation of cell growth using

STRING. The proteins like ADSS, ADSSL1, APRT, GMPR, GMPS, GUK1, IMPDH1, IMPDH2 and PNP were found to be associated with functional HPRT1 protein with a high confidence score (≥ 0.95) (Fig. 4). Most of these genes were found to be involved in purine nucleotide biosynthesis and regulation of cell growth. Any deleterious mutation in *HPRT1* gene might affect further protein interactions in the network and may lead to a cascading effect on the purine salvage pathway in bovines.

The current study reports the nine deleterious nsSNPs identified in the bovine *HPRT1* gene using *in silico* approach. The most deleterious SNP (rs465703426, G140V) revealed several structural changes in the HPRT protein leading to altered protein stability and energy change. The study infers that G140V may be regarded as a potential harmful SNP in bovine *HPRT1* gene, providing the evidence for *in vivo* and *in vitro* studies, further.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contributions: US, SKN: Conceived and designed the experiments; US, MM, GP: Performed the experiment; US, AK, YK, A: Wrote the paper; US, SKN: Revised the manuscript. All authors read and approved the final manuscript.

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