Biotechnology Journal International

24(2): 12-21, 2020; Article no.BJI.54823
ISSN: 2456-7051
(Past name: British Biotechnology Journal, Past ISSN: 2231–2927, NLM ID: 101616695)

In silico Analysis of Single Nucleotide Polymorphism in INHA Gene of Sheep and Goats

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Authors' contributions

This work was carried out in collaboration among all authors. Author RBF designed the study, wrote the protocol, 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.

Article Information

DOI: 10.9734/BJI/2020/v24i230098
Editor(s):
(1) Dr. Chung-Jen Chiang, China Medical University, Taiwan.
Reviewers:
(1) Nain Tara, Agha Khan University Hospital, Pakistan.
(2) Grace O. Tona, Ladoke Akintola University of Technology, Nigeria.
(3) Iwona Rybakowska, Medical University of Gdansk, Poland.
Complete Peer review History: http://www.sdiarticle4.com/review-history/54823

Original Research Article

Received 14 December 2019
Accepted 18 February 2020
Published 09 April 2020

ABSTRACT

Inhibin A (INHA) a member of the transforming growth factor-β (TGF-β) family has been implicated in the negative feedback control mechanism of the pituitary follicle stimulating hormone (FSH). Inhibin has been reported to be associated with litter size, milk yield, fertility and reproductive traits in ruminants.

A total of ten amino acid sequences (four sheep and six goats) were downloaded from the National Centre for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/snp). The amino acid sequence alignment was carried out using ClustalW algorithm of Molecular Evolutionary Genetic Analysis software version 6.0. The functional effects of eighteen (18) amino acid substitutions of INHA gene in each of sheep and goats were predicted computationally using Polyphen-2, PROVEAN and SIFT algorithms while INHA gene functions and interactions with associated genes were investigated using GeneMANIA. Variants that were consensually predicted to be deleterious by the three algorithms utilised were referred to as 'Cmutant' and 'Dmutant' in sheep and goats, respectively. The MutPred was further used to determine the tolerance degree for each amino acid substitution of both the 'Cmutant' and 'Dmutant'.

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GeneMANIA revealed 20 genes that co-localised, co-expressed with, play functionally similar role or has physical or genetic interaction with INHA gene. Out of the studied eighteen (18) amino acid substitutions; there was a consensus by SIFT, PROVEAN and PolyPhen-2 algorithms in the prediction of variants C99R, C264D, L237T and F14C as being deleterious in sheep and variants D77F, L190K, R223D, L240N, C322T and P326C in goats. In goats, MutPred revealed that variants R223D and D77F were not harmful while, L190K, R223D, L240N and P326C mutations were observed to be highly harmful. In sheep, the 'Dmutants' (C99R, C264D, F14C and L237T) were predicted to be highly harmful. The obtained findings would be useful in planning research aiming at exploring the association between INHA gene variants and economically important traits of small ruminants.

Keywords: Inhibin; goat; sheep; SNP; amino acid variants.

1. INTRODUCTION

Single nucleotide polymorphism (SNP) is the variation in a genetic sequence that affects only one of the basic building blocks of a DNA molecule and that occurs in more than 1% of a population [1]. The SNPs could be grouped into coding SNPs, noncoding SNPs, or the intergenic [2,3]. Unlike other SNPs, which are quite natural in the animal/human genome, the non-synonymous coding SNPs (nsSNPs) often have main impact on phenotype by changing the protein sequence via amino acid alteration in the corresponding protein product. It can also exert deleterious effects on the structure, function and stability of proteins or by modifying DNA and transcriptional binding factors and impacting the phenotype by changing the protein sequence [4,5]. Single nucleotide polymorphism in inhibin, MTNR1, PAPPA2, DGAT1 etc have been postulated to contribute or be associated with both economic and adaptive traits of farm animals including but not limited to disease resistance, longevity, milk yield, wool production, fertility traits, reproductive traits, laying performance and heat tolerance. Inhibins are dimericglyco proteins that inhibits pituitary follicle-stimulating hormone secretion, follicular maturation and steroidogenesis by suppressing its receptor expression in granulosa cells, thus affecting the development of ovarian follicles [6,7]. Inhibins are made up of dimmer of alpha and beta-A which commonly encoded by INHA, INHbA, and INHbB [8]. The gene has been physically mapped to chromosome 2q41-42 of Capra hircus INHA gene; consisting of three exons [9]. The transcript of goat INHA contains 1123 bp (NM_001285606), including a 1083 bp open reading frame, a 15-bp 5' UTR and a 25-bp 3' UTR.

In goats, [10] reported that the INHA 651A/G polymorphism was a potential marker for the mean litter size of the second parity in Boer goats. Earlier studies have demonstrated that immunization against inhibin could improve the ovarian response to superovulation, resulting in increased ovulation rates and higher yields of transferable embryos in sheep [11], heifers or cows [12,13], and water buffalo [14]. In this study, *in silico* analysis of the functional and structural effects of the genetic variants of INHA gene of sheep and goats were investigated. The observed beneficial SNPscan be used as marker to improve economically important traits in these farm animals.

2. MATERIALS AND METHODS

2.1 Sequence Retrieval and Analysis

The amino acid sequence data on sheep and goats INHA gene were retrieved from the database of the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/snp). A total of six (6) goat sequences and four (4) sheep sequences were downloaded. Details of the downloaded sequences are presented in Table 1. The amino acid sequence alignment of the two species was carried out using ClustalW algorithm of Molecular Evolutionary Genetic Analysis software version 6.0 [15].

2.2 Functional Prediction of Non-synonymous Amino Acid Substitutions

The functional effects of the nsSNPs of INHA gene in sheep and goats were predicted computationally using Polyphen-2, PROVEAN and SIFT while INHA gene functions and interactions with associated genes were investigated using GeneMANIA.

2.2.1 Investigation of INHA gene's interactions with functionally similar genes

GeneMANIA finds similar and functionally associated genes with the query gene using a wealth of genomics and proteomics data. The functionally related genes to INHA were obtained using the GeneMANIA (http://www.genemania.org). Genetic interactions, pathways, co-expression, co-localization and protein domain similarity of the INHA gene of sheep and goats were determined as previously described [16].

2.2.2 Prediction of structural impact of nsSNPs on protein by SIFT software

The Structural Impact of nsSNPs on the sheep and goats INHA gene sequences were predicted using SIFT (Separating Intolerant from Tolerant) software (http://sift.bii.a-star.edu.sg/.). SIFT is a sequence homology-based tool that uses multiple alignment information to predict tolerated and deleterious substitutions for each position of the query sequence. It first searches for related/similar sequences, then chooses closely functionally related sequences to the query sequence and finally calculates normalized probabilities for all possible substitutions from the alignment. Positions with normalized probabilities less than 0.05 were predicted to be deleterious while those greater than or equal to 0.05 were predicted to be tolerated [17].

2.2.3 Function analysis of nsSNP using provean

Protein Variation Effect Analyser (PROVEAN) was used to predict the single amino acids substitutions and functional effect of protein sequence variations. Variants with a PROVEAN score above -2.5 are considered "NEUTRAL" while variants with a PROVEAN score equal to or below -2.5 are considered "DELETERIOUS," [18].

2.2.4 Prediction of deleterious nsSNPs by polyphen-2

Polymorphism Phenotyping version 2.0 software available: http://genetics.bwh.harvard.edu/pph2/ (Polyphen-2) was also used to predict the possible impact of amino acid substitutions on the stability and function of INHA proteins using structural and comparative evolutionary considerations. Polyphen performs functional annotation of SNPs, maps coding SNPs to gene transcripts, estimates the probability of the missense mutation being damaging among others.

2.2.5 Prediction of harmful mutations by mutpred

The Mutpred server (http://mutpred.mutdb.org/) was employed to classify an amino acid substitution as deleterous or neutral. Additionally, Mutpred predicts molecular cause of deleterious amino acid sequence. The output of Mutpred contains the probability that the amino acid substitution is deleterious/disease-associated and top 5 property scores (p), where p is the P-value that certain structural and functional properties are impacted [19].

Table 1. Amino acid sequences of sheep and goats

Species	Accession number	Length (bp)
Goats	ABR13681.1	80
	NP_001272535.1	360
	ABR13682.1	263
	AEJ07666.1	360
	AEP40506.1	360
	AEP40507.1	360
Sheep	AIW82618.1	360
	ABS82446.1	265
	AAA31553.1	265
	NP_001295508.1	360

3. RESULTS AND DISCUSSION

Fertility is one of the most economically important traits in farm animal production. Genetic improvement of fertility traits in indigenous domestic animals will enhance productivity and food security especially in developing countries [18]. The INHA gene (Fig. 1) play a crucial role in fertility and reproductive rate in farm animals by suppressing the FSH receptors in the granular cells thereby affecting ovarian development. The INHA gene has been postulated to be associated with superovulation in small ruminants [20]. A total of ten amino acid sequences (six goats and 4 sheep) were retrieved from NCBI database. The sequence lengths for goats ranged between 80 and 360 while those of the sheep ranged between 265 and 360 base pairs.

GeneMANIA revealed vital functions of INHA gene as well as the genes that co-localised, co-expressed with, play functionally similar role or has physical or genetic interaction with INHA (Fig. 1). These include Follicle stimulating

Table 2. Gene description using GeneMania

SN	Genes	Description
1	INHA	Inhibin alpha subunit
2	FSHB	Follicle stimulating hormone beta subunit
3	CGA	Glycoprotein hormones, alpha polypeptide
4	ACVR2A	Activin A receptor type 2A
5	INHBB	Inhibin beta B subunit
6	PDIA3	Protein disulfide isomerase family A member 3
7	FST	Follistatin
8	INHBA	Inhibin beta A subunit
9	CALR	Calreticulin
10	MAPK4	Mitogen-activated protein kinase 4
11	IKBKG	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma
12	HMBOX1	Homeobox containing 1
13	CASP9	Caspase 9
14	TRIM29	Tripartite motif containing 29
15	BMP4	Bone morphogenetic protein 4
16	ALX4 ALX	Homeobox 4
17	ACVR1B	Activin A receptor type 1B
18	SERPINB3	Serpin family B member 3
19	GRAMD1B	GRAM domain containing 1B
20	INHBC	Inhibin beta C subunit
21	KCNQ1	Potassium voltage-gated channel subfamily Q member 1

hormone beta subunit, Glycoprotein hormones, alpha polypeptide, Activin A receptor type 2A, Protein disulfide isomerase family A member 3, Follistatin and Mitogen-activated protein kinase 4 (Table 2). This association further lay credence to the important role this gene plays in follicular development and oocyte maturation. Out of the studied eighteen (18) amino acid substitutions in each of sheep and goats; there was a consensus by SIFT, PROVEAN and PolyPhen-2 algorithms in the prediction of variants C99R, C264D, L237T and F14C as being deleterious in sheep (Table 3) and variants D77F, L190K, R223D, L240N, C322T and P326C in goats (Table 4). These ten variants (four in sheep and six in goats) were therefore collectively referred to as 'Cmutant' and 'Dmutant', respectively for further confirmatory analysis. Variants D201W, L50W, A43K, D244H, Y251D, and C227R were predicted deleterious by only 2 out of the three algorithms, W138A, L136V, L219A and F214Q were predicted deleterious by only one out of the three algorithms while E127H, H19Q, A225F and S248N were predicted to be tolerated by SIFT, PROVEAN and PolyPhen-2 algorithms in sheep. Similarly, Q124A, L148K, S200Y and E94Y were observed to be deleterious by two out of the three algorithms, A201V, T152S and S112A were predicted deleterious by only one while SIFT, PROVEAN and PolyPhen-2 algorithms predicted V301A, H128T, V63T, A176G and A100E to be neutral in goats. The differences in prediction capabilities refer to the fact that every prediction algorithm uses different sets of sequences and alignments [21]. The MutPred was further used to determine the tolerance degree for each amino acid substitution of both the 'Cmutant' and 'Dmutant' as described byHe et al. [22]. In goats, MutPred revealed that variants R223D and D77F were neutral while, L190K, R223D, L240N and P326C mutations were observed to be highly harmful. In sheep, the 'Dmutants' (C99R, C264D, F14C and L237T) were predicted to be highly harmful. This conforms with the earlier obtained results from SIFT, PROVEAN and PolyPhen-2 algorithms in this study. Details of the obtained results from MutPred are shown in Table 5. These results suggest that some nsSNPs such as the 'Cmuatnts' and 'Dmutants' may account for potential structural and functional changes in INHA protein. Similar observation was made by He et al. [22] in their study on insilico analysis of deleterious Single Nucleotide Polymorphisms (SNPs) in Human MutS Homolog6 (MSH6) gene. Earlier, [14] have identified polymorphism in INHA gene with a significant association with litter size in three goat breeds. Tian et al. [23] observed significantly different genotype distributions in INHA gene between year-round

estrus goat breeds and seasonal estrous goat breeds. Similarly, A282G mutation in INHA promoter had significant effects on the average litter size of Small Tailed Han sheep ($P < 0.05$) [24] and mutation homozygous genotypes had 1.32 lambs more than those with wild type in Small Tail Han Sheep [25]. In a similar study, [26] reported significant correlation between MspI polymorphism in the bovine INHA gene and superovulation. However, INHβA C7639T mutation has no significant effect on superovulation traits in Chinese Holstein cows. In exon 2, 651A/G and 125 G/A have been associated with litter size of the second parity in Boer, Matou and Nubi goats [10] and Dazu Black and Nanjiang Yellow goats, respectively [27]. However, exon 1 of INHA was conserved with no polymorphisms in goats [10,28,29]. This suggests possibility of INHA variants as marker against seasonal breeding in goats.

Table 3. The effect of amino acid variant on the functions of INHA proteins of sheep using PROVEAN, SIFT and polyphen-2

Amino Acid Change	SIFT prediction	SIFT score	PROVEAN Prediction	PROVEAN Score	PolyPhen -2 Prediction	PolyPhen-2 Score
D201W	Tolerated	0.68	Deleterious	-2.709	POROBABLY DAMAGING	0.976
E127H	Tolerated	0.68	Neutral	-1.619	POSSIBLY DAMAGING	0.865
H19Q	Tolerated	0.47	Neutral	-1.608	POROBABLY DAMAGING	0.999
F214Q	Tolerated	1.00	Neutral	-0.216	BENIGN	0.001
L219A	Tolerated	1.00	Neutral	0.108	BENIGN	0.046
Y251D	Tolerated	1.00	Deleterious	-5.654	POROBABLY DAMAGING	1.000
C99R	Deleterious	0.47	Deleterious	-4.627	POROBABLY DAMAGING	1.000
C264D	Deleterious	1.00	Deleterious	-9.257	POROBABLY DAMAGING	1.000
A225F	Tolerated	1.00	Neutral	-2.376	POSSIBLY DAMAGING	0.553
C227R	Tolerated	1.00	Deleterious	-10.169	PROBABLY DAMAGING	1.000
L237T	Deleterious	1.00	Deleterious	-4.237	POROBABLY DAMAGING	1.000
D244H	Tolerated	0.95	Deleterious	-5.413	PROBABLY DAMAGING	1.000
S248N	Tolerated	1.00	Neutral	-0.254	PROBABLY DAMAGING	1.000
F14C	Deleterious	0.47	Deleterious	-3.068	POROBABLY DAMAGING	1.000
L136V	Tolerated	0.84	Neutral	0.052	BENIGN	0.000
W138A	Tolerated	0.68	Neutral	-0.883	BENIGN	0.045
A43K	Deleterious	0.42	Neutral	-1.019	POSSIBLY DAMAGING	0.782
L50W	Deleterious	0.37	Neutral	-1.723	PROBABLY DAMAGING	0.997

PolyPhen-2 result: POROBABLY DAMAGING (more confident prediction) / POSSIBLY DAMAGING (less confident prediction), The amino acid substitution is predicted damaging if the score is ≤ 0.05, and tolerated if the score is > 0.05

Table 4. The effect of amino Acid variant on the functions of INHA proteins of Goats using PROVEAN, SIFT and PolyPhen-2

Amino Acid Change	PROVEAN Prediction	PROVEAN Score	PolyPhen-2 Prediction	PolyPhen-2 Score	SIFT Prediction	SIFT Score
A100E	Neutral	-0.342	Benign	0.002	Tolerated	0.15
E94Y	Neutral	-0.951	POSSIBLY DAMAGING	0.903	Deleterious	0.15
D77F	Deleterious	-2.587	PROBABLY DAMAGING	1.000	Deleterious	0.15
S112A	Neutral	-1.620	PROBABLY DAMAGING	1.000	Tolerated	0.15
T152S	Neutral	0.958	BENIGN	0.000	Deleterious	0.15
A176G	Neutral	-1.145	BENIGN	0.023	Tolerated	0.15
S200Y	Neutral	-0.827	PROBABLY DAMAGING	0.984	Deleterious	0.15
L190K	Deleterious	-3.134	PROBABLY DAMAGING	0.000	Deleterious	0.15
L148K	Neutral	-0.469	PROBABLY DAMAGING	1.000	Deleterious	0.15
Q124A	Neutral	-2.427	POSSIBLY DAMAGING	0.862	Deleterious	0.15
V63T	Neutral	0.158	BENIGN	0.002	Tolerated	0.15
H128T	Deleterious	-2.811	POSSIBLY DAMAGING	0.622	Deleterious	0.15
A201V	Neutral	-1.318	BENIGN	0.003	Deleterious	0.15
R223D	Deleterious	-4.297	PROBABLY DAMAGING	1.000	Deleterious	0.23
L240N	Deleterious	-2.578	PROBABLY DAMAGING	1.000	Deleterious	0.26
V301A	Neutral	0.131	BENIGN	0.001	Tolerated	0.83
C322T	Deleterious	-8.808	PROBABLY DAMAGING	1.000	Deleterious	0.85
P326C	Deleterious	-8.066	PROBABLY DAMAGING	0.999	Deleterious	0.85
A100E	Neutral	-0.342	BENIGN	0.002	Tolerated	0.15
E94Y	Neutral	-0.951	POSSIBLY DAMAGING	0.903	Deleterious	0.15
D77F	Deleterious	-2.587	PROBABLY DAMAGING	1.000	Deleterious	0.15

PolyPhen-2 result: POROBABLY DAMAGING (more confident prediction) / POSSIBLY DAMAGING (less confident prediction), The amino acid substitution is predicted damaging if the score is ≤ 0.05, and tolerated if the score is > 0.05

Table 5. Prediction of the functional impact of nsSNPS on INHA protein of sheep and goats by MutPred

Species	Amino acid change	Probability of deleterious mutation	Top 5 features	Affected PROSITE and ELM Motifs
Goats	R223D	0.428 (Not harmful)	None	None
	L190K	0.842 (Highly harmful)	Altered Stability (P = 0.0017) Gain of Strand (P = 0.0026) Loss of Loop (P = 0.0097)	ELME000045, ELME000148
	L240N	0.551 (Harmful)	Altered Disordered interface (P = 0.04) Gain of Loop (P = 0.02) Loss of Proteolytic cleavage at R241 (P = 0.02)	None
	D77F	0.245 (Not harmful)	None	None
	P326C	0.728 (Highly harmful)	Altered Metal binding (P = 0.01) Gain of Disulfide linkage at C321 (P = 0.00031) Gain of Helix (P = 0.04) Altered Transmembrane protein (P = 0.01) Gain of Catalytic site at C322 (P = 0.04)	ELME000336
Sheep	C99R	0.715 (Highly harmful)	Gain of Intrinsic disorder (P = 0.04) Loss of Strand (P = 0.05)	ELME000012, ELME000102, ELME000108, ELME000162
	C264D	0.922 (Highly harmful)	Gain of Intrinsic disorder (P = 0.000073) Altered Metal binding (P = 0.00046) Altered Transmembrane protein (P = 0.00) Gain of Relative solvent accessibility (P = 0.0001) Altered Ordered interface (P = 0.0017) Altered Disordered interface (P = 0.02) Gain of Catalytic site at C264 (P = 0.0046) Gain of Disulfide linkage at C262 (P = 0.03)	None
	F14C	0.864 (Highly harmful)	Altered Transmembrane protein (P = 0.03)	ELME000155, ELME000163, ELME000182, ELME000202, ELME000328
	L237T	0.845 (Highly harmful)	Altered Transmembrane protein (P = 0.00015) Gain of ADP-ribosylation at R235 (P = 0.03) Altered Stability (P = 0.04)	ELME000012, ELME000053, ELME000062, ELME000106

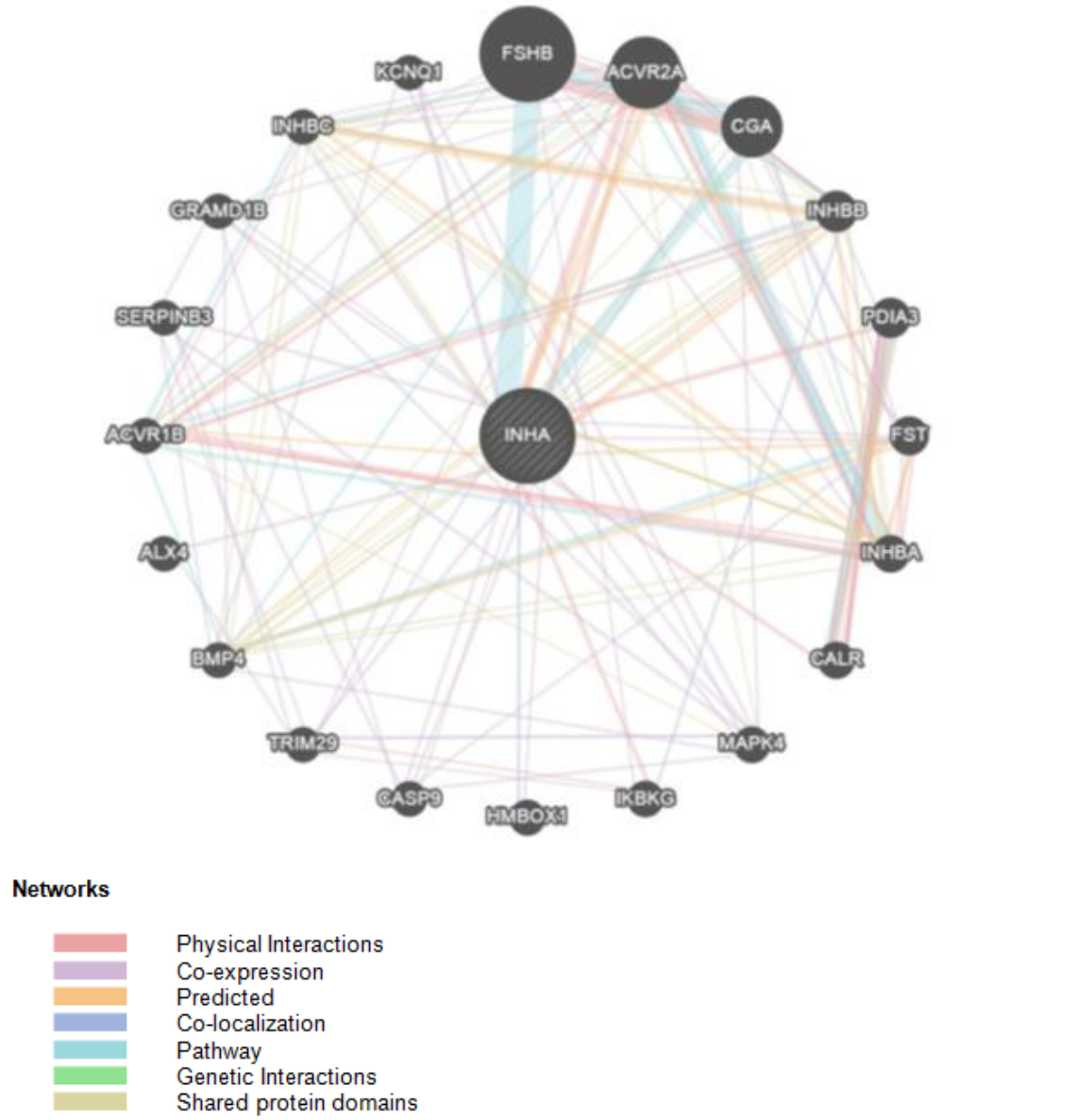

Fig. 1. GeneMania derived functional association between INHA and other related genes

4. CONCLUSION

This study provides information on the potential deleterious effects of single nucleotide polymorphism on functions and structures of INHA gene of sheep and goats. All the four nsSNPS that were consensually predicted to be the deleterious by SIFT, PROVEAN and PolyPhen-2 were also observed to be deleterious by Mutpred in sheep, while R223D and D77F were observed to be neutral in goats after been predicted to be deleterious by the three software.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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