Leptin Signaling: Decoding of Genetic Pathways using Bioinformatics; Shaping Bariatric Surgery Outcomes

Usha Adiga[1], Sampara Vasishta[1,*] and Alfred J. Augustine[2]

[1]*Department of Biochemistry, Apollo Institute of Medical Sciences and Research, Murukambattu - 517127, Chittoor, Andhra Pradesh, India*

[2]*Department of Surgery, Apollo Institute of Medical Sciences and Research, Murukambattu - 517127, Chittoor, Andhra Pradesh, India*

Abstract: *Background*: Leptin, a hormone central to energy homeostasis and appetite regulation, plays a pivotal role in obesity and metabolic health. Single nucleotide polymorphisms (SNPs) in the leptin (*LEP*) and leptin receptor (*LEPR*) genes influence leptin signaling and may explain variability in outcomes following bariatric surgery. This bioinformatics-driven study examines the role of *LEP* and *LEPR* SNPs in modulating weight loss, metabolic changes, and hormonal responses post-surgery.

Methods: A total of 55 leptin SNPs and 216 leptin receptor SNPs were assessed for functional impact using SIFT, PolyPhen-2, and Mutation Assessor. Pathway enrichment analyses using DAVID and g:Profiler identified biological processes and signaling pathways linked to leptin function. Protein-protein interaction (PPI) networks were constructed via STRING and visualized in Cytoscape to explore molecular interactions. Statistical models evaluated associations between SNPs and surgical outcomes, including weight loss and metabolic improvements. Key pathways with false discovery rates (FDR) < 0.01 were highlighted to emphasize significance.

Results: Bioinformatics analyses revealed *LEP* and *LEPR* as critical variants associated with bariatric surgery outcomes. Specifically, *LEP* rs7799039 G allele carriers exhibited diminished weight loss ($p < 0.05$) and metabolic improvements. Functional prediction tools consistently indicated deleterious effects on leptin signaling. Pathway enrichment analyses identified leptin's involvement in critical pathways, including the adipocytokine signaling pathway (hsa04920, 2 of 68 genes, strength = 2.46, FDR = 0.0042)," "AMPK signaling pathway (hsa04152, 2 of 120 genes, strength = 2.22, FDR = 0.0064)," and "non-alcoholic fatty liver disease (NAFLD) pathway (hsa04932, 2 of 146 genes, strength = 2.13, FDR = 0.0064). PPI networks underscored leptin's interactions with key metabolic and inflammatory regulators, such as TNF-α and IL-6, suggesting a broader impact on energy metabolism and inflammation.

Conclusion: This study demonstrates the utility of bioinformatics in elucidating the genetic basis of variable bariatric surgery outcomes. *LEP* and *LEPR* SNPs modulate critical pathways influencing weight loss and metabolic responses. Integrating genetic insights with bariatric care could advance precision medicine approaches for obesity management. Future studies with larger cohorts are warranted to confirm these findings and strengthen predictive models.

Keywords: Leptin, leptin receptor, bioinformatics, SNPs, bariatric surgery, weight loss, pathway enrichment.

INTRODUCTION

Obesity is a major global health challenge, characterized by significant comorbidities such as type 2 diabetes, cardiovascular disease, and metabolic syndrome [1]. Bariatric surgery remains the most effective treatment for severe obesity, achieving sustained weight loss and improved metabolic outcomes. However, postoperative responses vary widely, driven in part by genetic factors that influence metabolic regulation and hormonal adaptations.

Leptin, a hormone secreted by adipocytes, regulates appetite, energy expenditure, and metabolism. Leptin exerts its effects through its receptor (*LEPR*), primarily in the hypothalamus, modulating satiety and energy homeostasis. Genetic variations in the leptin (*LEP*) and leptin receptor (*LEPR*) genes are known to affect leptin signaling, influencing obesity susceptibility and the efficacy of weight loss interventions. Single nucleotide polymorphisms (SNPs) in these genes, have been linked to altered leptin expression and receptor functionality, respectively.

This study employs bioinformatics approaches to investigate the functional and biological implications of *LEP* and *LEPR* SNPs in the context of bariatric surgery outcomes. By integrating computational predictions, pathway enrichment, and protein-protein interaction (PPI) analyses, we aim to elucidate how these genetic variations influence weight loss, metabolic changes, and hormonal responses. Our findings provide a foundation for leveraging genetic data in personalized obesity management.

SNPs (single nucleotide polymorphisms) are variations in a single nucleotide that result in alterations to the DNA sequence (A, T, C, or G). SNPs make up about 90% of the total genetic diversity in humans. The

*Address correspondence to this author at the Department of Biochemistry, Apollo Institute of Medical Sciences and Research, Murukambattu - 517127, Chittoor, Andhra Pradesh, India; E-mail: vasishta_s@aimsrchittoor.edu.in

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3-billion-base-long human genome contains SNPs at intervals of 100–300 bases, with varying density in various regions [2]. Both coding and noncoding regions of the genome are susceptible to SNPs. SNPs can have a variety of outcomes, ranging from having no impact on cellular function to causing disease or changing how a medicine interacts with the body. The fact that nonsynonymous SNPs (nsSNPs), which produce an amino acid residue substitution in the protein product, account for almost half of all genetic variations linked to inherited disease in humans, makes them particularly important [3]. Coding synonymous SNPs (sSNPs), as well as non-coding SNPs (sSNPs), can nevertheless have an impact on transcription factor binding, splicing, and gene expression [4,5].

SNPs must be found because they cause particular traits, making their detection essential. This is a challenging undertaking because it calls for the assessment of tens of thousands of SNPs in potential genes [6]. Selecting which SNPs to include in a study is a challenging decision whenever a study is being conducted to examine the significance of an SNP in disease. In such circumstances, separating functional from neutral SNPs may be possible using bioinformatics prediction algorithms. They might also reveal the structural basis of the mutations. Simply put, these bioinformatics tools are ways to order SNPs according to their functional significance [7,8].

By using bioinformatics techniques for *In silico* gene analysis, it is no longer necessary to screen a huge number of people in order to identify a gene-disease association with a sufficient level of statistical significance. In other words, these techniques support SNP pre-selection [6].

Before using wet lab-based approaches, it would be very helpful if disease-associated SNPs could be separated from neutral SNPs. *In silico* analyses are helpful when the disease connections could not be established by future independent research [7]. As a result, additional resources could be employed to distinguish between true and false positives by using independent proof of SNP functionality discovered by the application of prediction algorithms.

By employing bioinformatics methods for *In silico* gene analysis, it is possible to detect a link between a gene and a disease at a level of statistical significance without screening a sizable number of people. In other words, these tools help in the pre-selection of SNPs.

The aim of the study is to carry out the *In silico* analysis of leptin and its receptor gene using bioinformatics tools such as sorting the intolerant from tolerant (SIFT), Provean and I-mutant softwares.

The novelty of this study lies in its integrative bioinformatics approach, which combines computational predictions (SIFT, Provean, and I-Mutant), pathway enrichment, and protein-protein interaction (PPI) analyses to pre-select functional SNPs with potential disease relevance. This method minimizes the need for large-scale screening and enhances the identification of genetic factors influencing bariatric surgery outcomes.

METHODOLOGY

The research evaluated genetic differences in the leptin (*LEP*) and leptin receptor (*LEPR*) genes employing bioinformatics methodologies. A total of 267 nonsynonymous single nucleotide polymorphisms (nsSNPs) were discovered, comprising 55 from *LEP* and 216 from *LEPR*. Functional predictions indicated that 161 nsSNPs were detrimental (by SIFT), 77 were harmful (via PROVEAN), and 251 demonstrated reduced protein stability (via I-Mutant), suggesting substantial effects on leptin and leptin receptor activities.

The analysis of *LEP* and *LEPR* genes using bioinformatics tools is depicted in Figure **1**:

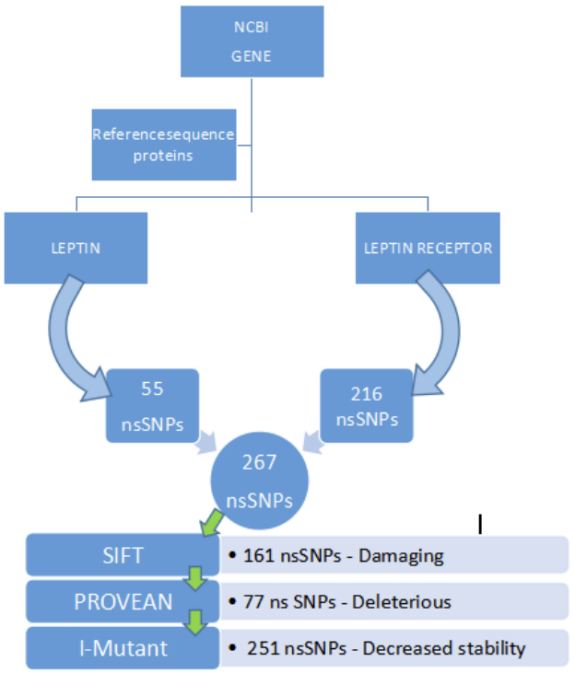

Figure 1: Depicting the analysis of genes using bioinformatics tools.

Evaluation of the Functional Impact of Coding nsSNPs Using a Sequence Homology Tool sorting intolerant from tolerant (SIFT):

To forecast tolerated and harmful substitutions at each place in the query sequence, SIFT (http://sift.jcvi.org) analyses the query sequence and makes use of various alignment information [9]. It is a multi-step process that, given a protein sequence, first looks for related sequences, then chooses closely related sequences that might have similar functions, then obtains multiple alignments of these selected sequences, and finally calculates normalised probabilities for all potential substitutions at each position from the alignment. Those substitutions with normalised probabilities more than or equal to 0.05 are predicted to be tolerated, while those with normalised probabilities less than 0.05 are predicted to be harmful [10].

By letting the algorithm search for homologous sequences using its default settings, the investigation was conducted (UniProt-TrEMBL 39.6 database, median conservation of sequences of 3.00, and allowance to remove sequences more than 90 percent identical to query sequence). The SIFT approach ascertains if alterations of amino acids affect how proteins function. It functions by utilising the physical-chemical properties of amino acid residues as well as sequence homology between related genes and domains. Using the web programme Sort the Intolerant from Tolerant, the total numbers of non-intronic missense mutations, rs numbers, and the locations of SNPs on chromosomes for leptin and leptin receptor were recorded in a format suitable for analysis (SIFT). The FASTA amino acid sequence of the NCBI Protein accession id NP_000221 for leptin gene and NP_002294.2 for leptin receptor were used as the query sequence, and filtered nsSNPs from the dbSNP database were analyzed.

Evaluation of the Functional Impact of Coding nsSNPs Using Provean

Although PROVEAN is a popular bioinformatic tool for summarising the health of various populations according to their mutations, no attempts have been made to validate its predictions at the genome level. The Protein Variant Effect Analyzer (PROVEAN), developed by Choi *et al*., forecasts the effects of in-frame insertions and deletions in addition to amino acid substitutions [11]. SIFT and PolyPhen-2, which use sequence comparisons from BLAST searches and are hence dependent on the database selection, work in a manner that is similar to PROVEAN's [11,12]. PROVEAN collects groups of highly similar sequences from the NCBI nonredundant protein sequences(nr) database, much like SIFT does.

PROVEAN calculates an alignment score for both the query sequence (i.e., the wild type) and the mutant to these sequence clusters rather than producing probabilities of substitution across the protein of interest. The PROVEAN score is the difference between the mean alignment scores for the query and mutant proteins. Protein alignment in PROVEAN uses the BLOSUM62 matrix, which has blocks aligned from proteins that are fewer than 62 percent identical. Only the conserved sections of these proteins are employed in the BLOSUM matrix, guaranteeing that their similarities and differences indicate selection, or lack thereof. A 62 percent cut-off assures that the proteins that are being compared are divergent. Using the given query sequence, a BLAST [13,14] search is conducted as the initial phase of PROVEAN. For the purpose of identifying homologous but yet distantly related sequences, an Expect value cut-off of 0.1 is employed. This usually yields thousands of matches for a variety of taxa. These sequences are grouped based on a cutoff of 75 percent sequence similarity within a cluster to prevent duplication. The alignment scores to the query and mutant sequences, as well as the PROVEAN score, are then calculated for the top 30 clusters that are most similar to the query sequence. The supporting sequence set may be independently preserved and analysed. The computer reports a predicted functional category, either harmful or neutral, based on the PROVEAN score and a predetermined threshold. There is no category for advantageous impacts, even though it is feasible for a mutant protein to have a higher mean alignment score than the wild type. Variants with scores below the default cutoff value of 2.5 are categorised as harmful. This cutoff was established to maximise sensitivity and specificity for determining which human protein variations commonly cause disease and which have functional effects [15].

Evaluation of the Functional Impact of Coding nsSNPs Using I mutant 3.0

I-Mutant 3.0 is a support vector machine (SVM)-based tool for the automatic prediction of protein stability changes upon single point mutations. I-Mutant 3.0 predictions are performed starting either from the protein structure or, more importantly, from the protein sequence.

In all the three tools, SIFT, Provean and I mutant, amino acid sequence obtained by the protein accession IDs were used for the analysis.

Pathway Enrichment: Gene Ontology (GO) and KEGG pathway analyses were performed using:

- **DAVID (v6.8)**: To identify enriched biological processes and functions.
- **g:Profiler**: To explore pathways associated with leptin and its receptor.

PPI Networks: Interaction networks were constructed using STRING (v11.5) and visualized in Cytoscape. Nodes and edges represented proteins and their interactions, respectively, highlighting leptin's role in metabolic and inflammatory pathways.

RESULTS

SIFT Analysis of leptin Gene showed that coding variants were 100%, but predicted ones were 96% (53 of 55), tolerated were 75% (40/53), damaging were 25% (13/53), 96% (53 of 55) were non-synonymous and only 4%(2 of 55) were synonymous. Eighty-three percent (46 of 55) of them were novel. SIFT score varies from 0-1.SNPs with SIFT score of less than or equal to 0.05 is considered to be damaging, above that is taken to be tolerant. Median info ranges from 0-4.32,ideally between 2.75-3.5.This is used to measure the diversity of the sequences used for prediction. A value greater than 3.25 indicates warning suggesting that the prediction was based on closely related sequences. Sequences at position is the number of sequences that have an amino acid at the position of prediction. SIFT chooses sequences automatically, but if the substitution is located at the beginning or end of the protein, there may be only few sequences represe-nted at that position and this column indicates this fact.

In this context, it is important to note that specific statistical measures such as odds ratios or effect sizes cannot be attributed, as this analysis is bioinformatics-based. The focus of bioinformatics analyses typically revolves around identifying associations and patterns within large datasets, rather than directly estimating clinical or biological metrics like odds ratios or effect sizes, which are more commonly used in clinical trials or experimental studies. Thus, the findings from bioinformatics analyses are generally indicative of correlations or potential associations that warrant further validation through experimental or clinical research.

Provean scores of the selected SNPs lesser than -2.5 suggested neutral mutations. A total of 17 (31%) mutations were deleterious and 38 (69%) were neutral. The number of SNPs found to be deleterious by Provean analysis is more than that obtained by SIFT analysis. This could be the due to the fact that Provean tool can analyze even insertions and deletions in addition to amino acid substitutions.

On I mutant suite 3.0 analysis, DDG values of binary classification of SNPs of genes showing values <0 implied a decreased stability. A difference in free energy, called delta G (ΔG) or DDG, is involved in each chemical reaction. For any mechanism which undergoes a transition, such as a chemical reaction, the change in free energy can be determined. Out of 55 SNPs, 47(85%) showed a decreased stability and only 8 (15%) alleles showed increased stability after mutation. This analysis suggested that majority of the mutations, irrespective of whether deleterious or neutral, resulted in decreased protein stability.

SIFT Analysis of Leptin R Gene

Missense mutations were filtered for leptin receptor gene and a total of 216 SNPs were detected. Hundred percent were coding variants, coding variants predicted were 98% (212 of 216), 31% of which were tolerated (64 of 212), 69% (148 of 212) were damaged, 98% (212 of 216) were non-synonymous and 2% (4 of 216) were synonymous. 84% (183 of 216) SNPs were novel. On Provean analysis, 39 of 216 SNPs (18%) were deleterious whereas 177 were neutral (82%). On I mutant analysis, 204 SNPs (94.4%) resulted in decreased stability and only 12 mutations (5.5%) resulted in increased stability.

Protein-protein interactions of leptin-leptin receptor are as depicted in Figure **2**.

The interaction between leptin and its receptor (*LEPR*) forms a simple but critical protein-protein interaction network, consisting of 2 nodes and 1 direct edge. This interaction highlights the direct signalling relationship between leptin and *LEPR*, which is central to regulating energy homeostasis, appetite, and metabolism. The network's average node degree of 1 reflects a straightforward one-to-one interaction, with no additional nodes or connections, and an average local clustering coefficient of 1 indicates that all nodes are maximally interconnected within this minimal network. Although the expected number of edges in this network is 0 due to its simplicity, the observed

Table 1: Analysis of SNPs of Leptin Gene with Bioinformatics Tool

Coordinates	Provean score	Provean Prediction	SIFT Score	SIFT Prediction	Median Info	SVM2 Prediction Effect (Kcal/mol)	DDG Value Prediction
7,127892205,1,T/A	-2.582	Deleterious	0.02	DAMAGING	3.01	-2.08	Decrease
7,127894580,1,C/A	-5.385	Deleterious	0.06	TOLERATED	2.9	-1.26	Decrease
7,127894551,1,C/T	-2.608	Deleterious	0.11	TOLERATED	2.9	0.21	Increase
7,127894767,1,G/A	-0.849	Neutral	0.1	TOLERATED	2.98	-0.08	Decrease
7,127894631,1,C/G	-2.373	Neutral	0	DAMAGING	2.91	-1.47	Decrease
7,127894467,1,C/T	-3.678	Deleterious	0.02	DAMAGING	2.91	0.13	Increase
7,127892101,1,G/C	-1.538	Neutral	0.14	TOLERATED	2.96	-0.93	Decrease
7,127894677,1,C/A	-2.015	Neutral	0.06	TOLERATED	2.92	-0.26	Decrease
7,127894692,1,C/A	-1.17	Neutral	0.2	TOLERATED	2.92	-0.68	Decrease
7,127894794,1,T/G	-4.036	Deleterious	0	DAMAGING	2.98	-1.21	Decrease
7,127894802,1,A/C	-1.614	Neutral	0.43	TOLERATED	2.98	0.35	Increase
7,127892214,1,C/G	-0.754	Neutral	0.86	TOLERATED	3.01	-0.62	Decrease
7,127892105,1,C/A	-0.51	Neutral	0.17	TOLERATED	2.96	-0.8	Decrease
7,127892117,1,C/G	-1.15	Neutral	0.29	TOLERATED	2.91	-1.39	Decrease
7,127894699,1,C/G	1.098	Neutral	1	TOLERATED	2.92	-0.02	Decrease
7,127892115,1,A/G	-0.053	Neutral	0.19	TOLERATED	2.91	-1.11	Decrease
7,127894808,1,G/A	-2.761	Deleterious	0.1	TOLERATED	2.99	-0.07	Decrease
7,127894710,1,G/A	-1.031	Neutral	0.84	TOLERATED	2.92	-0.75	Decrease
7,127894475,1,C/A	-1.804	Neutral	1	TOLERATED	2.91	-0.25	Decrease
7,127894721,1,G/A	-0.525	Neutral	0.28	TOLERATED	2.92	-0.67	Decrease
7,127894639,1,C/A	-2.92	Deleterious	0.35	TOLERATED	2.9	-0.25	Decrease
7,127894766,1,G/C	-0.57	Neutral	0.09	TOLERATED	2.98	-0.25	Decrease
7,127894550,1,G/T	-1.487	Neutral	0.13	TOLERATED	2.9	-0.65	Decrease
7,127894778,1,G/A	-1.215	Neutral	0.45	TOLERATED	2.98	-0.53	Decrease
7,127892169,1,C/A	-1.255	Neutral	0.14	TOLERATED	2.91	-0.81	Decrease
7,127894499,1,A/C	-1.38	Neutral	0	DAMAGING	2.9	-0.87	Decrease
7,127894568,1,C/T	-3.303	Deleterious	0.06	TOLERATED	2.9	-1.03	Decrease
7,127894793,1,C/G	-2.018	Neutral	0	DAMAGING	2.98	-1.04	Decrease
7,127894586,1,A/C	0	Neutral	N/A	N/A	N/A	-	-
7,127894610,1,G/A	-4.797	Deleterious	0	DAMAGING	2.9	-1.29	Decrease
7,127894765,1,G/A	0	Neutral	N/A	N/A	N/A	-	-
7,127894660,1,C/G	-2.196	Neutral	0.04	DAMAGING	2.92	0.03	Increase
7,127892199,1,A/C	-2.325	Neutral	0.45	TOLERATED	2.91	0.04	Increase
7,127894769,1,T/A	-0.637	Neutral	0.41	TOLERATED	2.98	-0.07	Decrease
7,127894655,1,A/G	-2.185	Neutral	0.08	TOLERATED	2.92	-0.03	Decrease
7,127892120,1,T/C	-0.27	Neutral	0.65	TOLERATED	2.92	-1.04	Decrease
7,127894538,1,G/T	-5.69	Deleterious	0.12	TOLERATED	2.9	0.45	Increase
7,127894804,1,C/A	-1.614	Neutral	0.43	TOLERATED	2.98	0.35	Increase
7,127894487,1,G/A	-4.931	Deleterious	0	DAMAGING	2.9	-1.28	Decrease
7,127894706,1,G/C	-2.816	Deleterious	0.22	TOLERATED	2.92	-0.45	Decrease

(Table 1). Continued.

Coordinates	Provean score	Provean Prediction	SIFT Score	SIFT Prediction	Median Info	SVM2 Prediction Effect (Kcal/mol)	DDG Value Prediction
7,127894679,1,A/G	-1.485	Neutral	0.7	TOLERATED	2.92	-0.92	Decrease
7,127892129,1,C/G	-0.175	Neutral	0.56	TOLERATED	2.92	0	Increase
7,127892093,1,G/A	0.379	Neutral	0.97	TOLERATED	2.95	-0.15	Decrease
7,127894574,1,A/T	-3.881	Deleterious	0	DAMAGING	2.91	-0.45	Decrease
7,127894800,1,T/G	-0.948	Neutral	0.43	TOLERATED	2.98	-1.21	Decrease
7,127894796,1,G/A	-2.446	Neutral	0.19	TOLERATED	2.98	-0.51	Decrease
7,127892124,1,A/G	-2.899	Deleterious	0.21	TOLERATED	2.92	-0.99	Decrease
7,127892204,1,A/G	-0.487	Neutral	0.2	TOLERATED	3.01	-1.19	Decrease
7,127892178,1,A/G	-1.285	Neutral	0.48	TOLERATED	2.91	-0.26	Decrease
7,127894625,1,C/T	-5.268	Deleterious	0	DAMAGING	2.9	-0.42	Decrease
7,127892109,1,G/T	-4.119	Deleterious	0.39	TOLERATED	2.96	-0.84	Decrease
7,127894792,1,G/C	-2.155	Neutral	0.2	TOLERATED	2.98	-0.51	Decrease
7,127894621,1,C/A	-5.228	Deleterious	0	DAMAGING	2.9	-0.45	Decrease
7,127894592,1,G/A	-1.529	Neutral	0.25	TOLERATED	2.9	-0.9	Decrease
7,127894640,1,G/A	0.914	Neutral	0.03	DAMAGING	2.9	-0.89	Decrease

Table 2: Analysis of Leptin R Gene by Bioinformatics Tools

Coordinates	Provean score	Provean Prediction	SIFT Score	SIFT Prediction	Median Info	SVM2 Prediction Effect (Kcal/mol)	DDG Value Prediction
1,66058477,1,T/C	-1.492	Neutral	0.02	DAMAGING	2.8	-0.77	Decrease
1,66075978,1,A/C	-4.553	Deleterious	0.01	DAMAGING	2.79	-0.1	Decrease
1,66102177,1,T/C	-1.296	Neutral	0.12	TOLERATED	2.83	-0.19	Decrease
1,66036303,1,A/G	0.085	Neutral	0.61	TOLERATED	2.79	-0.24	Decrease
1,66074534,1,C/T	-3.113	Deleterious	0.01	DAMAGING	2.79	-1.24	Decrease
1,66074527,1,G/C	-1.196	Neutral	0.43	TOLERATED	2.79	-0.34	Decrease
1,66102373,1,A/G	-	-	0.29	TOLERATED	2.83	-1.03	Decrease
1,66067233,1,A/G	-1.502	Neutral	0.52	TOLERATED	2.79	-1.2	Decrease
1,66085678,1,A/G	0	Neutral	N/A	N/A	N/A	-	-
1,66067246,1,G/A	-2.549	Deleterious	0	DAMAGING	2.79	-0.67	Decrease
1,66031263,1,A/C	-0.855	Neutral	0.22	TOLERATED	3.03	-0.13	Decrease
1,66087080,1,G/A	-0.562	Neutral	0.04	DAMAGING	2.79	-1.08	Decrease
1,66038105,1,A/G	-0.046	Neutral	0.04	DAMAGING	2.79	-0.73	Decrease
1,66064428,1,G/C	-1.391	Neutral	0.17	TOLERATED	2.87	-0.95	Decrease
1,66102507,1,C/T			0.21	TOLERATED	2.84	-0.81	Decrease
1,66088608,1,G/A	-0.128	Neutral	0.38	TOLERATED	2.79	-0.68	Decrease
1,66102637,1,A/C			0.03	DAMAGING	2.84	-1.51	Decrease
1,66038129,1,T/G	-1.312	Neutral	0.1	TOLERATED	2.79	-2.68	Decrease
1,66036177,1,C/T	-1.535	Neutral	0.01	DAMAGING	2.99	0.05	Increase
1,66102658,1,A/G			0.21	TOLERATED	2.84	-1.25	Decrease
1,66067543,1,T/C	-2.821	Deleterious	0.02	DAMAGING	2.79	-0.38	Decrease

(Table 2). Continued.

Coordinates	Provean score	Provean Prediction	SIFT Score	SIFT Prediction	Median Info	SVM2 Prediction Effect (Kcal/mol)	DDG Value Prediction
1,66081817,1,C/G	0.462	Neutral	1	TOLERATED	2.79	0.15	Increase
1,66062238,1,G/A	-1.87	Neutral	0.01	DAMAGING	2.87	-1.33	Decrease
1,66101967,1,A/G	-1.661	Neutral	0.04	DAMAGING	2.8	-1	Decrease
1,66064446,1,T/C	-1.669	Neutral	0.25	TOLERATED	2.87	-2.29	Decrease
1,66102217,1,A/G	-1.337	Neutral	0.65	TOLERATED	2.83	-0.41	Decrease
1,66083790,1,C/A	-0.278	Neutral	0.41	TOLERATED	2.79	-1.11	Decrease
1,66058363,1,C/G	-1.661	Neutral	0.08	TOLERATED	2.79	-0.83	Decrease
1,66088623,1,C/T	-4.801	Deleterious	0	DAMAGING	2.79	-1.52	Decrease
1,66087109,1,A/T	-2.353	Neutral	0.01	DAMAGING	2.79	-0.96	Decrease
1,66075636,1,G/A	-1.344	Neutral	0.15	TOLERATED	2.79	-0.67	Decrease
1,66102668,1,G/A	-	-	0.53	TOLERATED	2.84	0.33	Increase
1,66036191,1,T/C	-1.171	Neutral	0.21	TOLERATED	2.99	-1.63	Decrease
1,66038090,1,T/C	1.468	Neutral	0.16	TOLERATED	2.79	-1.89	Decrease
1,66067562,1,A/G	-3.267	Deleterious	0.04	DAMAGING	2.79	-1.18	Decrease
1,66102067,1,G/A	-	-	0.18	TOLERATED	2.83	-	-
1,66081833,1,C/T	-	-	0.04	DAMAGING	2.79	-0.02	Decrease
1,66101982,1,T/C	-2.181	Neutral	0.52	TOLERATED	2.8	-1.07	Decrease
1,66036465,1,C/A	-1.183	Neutral	0.11	TOLERATED	2.87	-0.12	Increase
1,66102355,1,C/T			0.09	TOLERATED	2.99	-0.48	Decrease
1,66070864,1,A/G	-0.197	Neutral	0.52	TOLERATED	2.79	-0.49	Decrease
1,66102382,1,T/G	-	-	0.02	DAMAGING	2.83	0.51	Increase
1,66087104,1,A/G	-0.179	Neutral	1	TOLERATED	2.79	-0.67	Decrease
1,66075697,1,A/C	-7.205	Deleterious	0	DAMAGING	2.79	-1.24	Decrease
1,66075945,1,T/C	0.661	Neutral	0.66	TOLERATED	2.79	-1.03	Decrease
1,66036249,1,A/T	-1.915	Neutral	0.04	DAMAGING	3.05	0.01	Increase
1,66102569,1,T/C	-	-	N/A	N/A	N/A	-0.75	Decrease
1,66058399,1,A/G	-0.448	Neutral	0.71	TOLERATED	2.8	0.14	Increase
1,66058536,1,C/T	-3.603	Deleterious	0.03	DAMAGING	2.79	-1.36	Decrease
1,66102159,1,G/A			0	DAMAGING	2.83	-0.49	Decrease
1,66083827,1,A/G	-1.209	Neutral	0.16	TOLERATED	2.79	-0.22	Decrease
1,66070774,1,A/G	-1.501	Neutral	0.11	TOLERATED	2.79	-0.39	Decrease
1,66101994,1,G/A	-0.828	Neutral	0.32	TOLERATED	2.81	-0.79	Decrease
1,66062268,1,A/T	-0.64	Neutral	0.11	TOLERATED	2.87	-1.55	Decrease
1,66081859,1,G/C	-1.391	Neutral	0.22	TOLERATED	2.79	-0.4	Decrease
1,66062157,1,C/T	-1.7	Neutral	0.95	TOLERATED	2.87	0.04	Increase
1,66070792,1,G/A	-0.384	Neutral	0.5	TOLERATED	2.79	-0.41	Decrease
1,66102423,1,A/G			0.08	TOLERATED	2.83	-0.36	Decrease
1,66067264,1,A/G	-2.046	Neutral	0.09	TOLERATED	3.01	-0.5	Decrease
1,66081883,1,A/G	-3.478	Deleterious	0	DAMAGING	2.79	-1.5	Decrease
1,66067150,1,A/G	-2.411	Neutral	0.24	TOLERATED	2.99	-0.93	Decrease
1,66101920,1,C/T	-1.561	Neutral	0.15	TOLERATED	2.8	-0.11	Decrease

(Table 2). Continued.

Coordinates	Provean score	Provean Prediction	SIFT Score	SIFT Prediction	Median Info	SVM2 Prediction Effect (Kcal/mol)	DDG Value Prediction
1,66064344,1,C/T	0.309	Neutral	1	TOLERATED	2.87	-0.01	Decrease
1,66075673,1,C/T	-0.126	Neutral	0.33	TOLERATED	2.79	-0.81	Decrease
1,66067167,1,T/C	-2.905	Deleterious	0.02	DAMAGING	2.79	-0.27	Decrease
1,66081728,1,G/C	-1.344	Neutral	0.44	TOLERATED	2.79	-1.03	Decrease
1,66067285,1,G/A	-0.812	Neutral	0.11	TOLERATED	2.8	-1.16	Decrease
1,66070741,1,A/G	-2.477	Neutral	0.08	TOLERATED	2.79	-1.16	Decrease
1,66038028,1,G/A	0	Neutral	N/A	N/A	N/A	-	-
1,66102148,1,T/A	-	-	0.16	TOLERATED	2.83	-1.3	Decrease
1,66064358,1,T/A	-0.88	Neutral	0.3	TOLERATED	2.87	-0.36	Decrease
1,66036222,1,C/A	-1.715	Neutral	0.06	TOLERATED	2.99	-0.17	Decrease
1,66036356,1,A/G	0.476	Neutral	0.91	TOLERATED	2.93	-0.39	Decrease
1,66038014,1,A/G	-1.025	Neutral	0.3	TOLERATED	2.87	-0.02	Decrease
1,66083698,1,T/C	-1.037	Neutral	0.01	DAMAGING	2.79	-2.35	Decrease
1,66081727,1,A/G	1.724	Neutral	0.48	TOLERATED	2.79	-1.66	Decrease
1,66102220,1,G/A	-0.85	Neutral	0.1	TOLERATED	2.83	-0.64	Decrease
1,66081829,1,G/A	-0.685	Neutral	0.26	TOLERATED	2.79	-0.28	Decrease
1,66083800,1,C/A	-2.392	Neutral	0	DAMAGING	2.79	-0.29	Decrease
1,66075670,1,T/C	-3.092	Deleterious	0	DAMAGING	2.79	-1.5	Decrease
1,66074584,1,G/A	0	-	N/A	N/A	N/A	-	-
1,66036212,1,T/C	-2.139	Neutral	0	DAMAGING	2.99	-1.08	Decrease
1,66064469,1,C/T	-0.559	Neutral	0.17	TOLERATED	2.87	-0.9	Decrease
1,66102129,1,G/A	-	-	0.52	TOLERATED	2.83	-1.17	Decrease
1,66102550,1,A/C	-	-	0.01	DAMAGING	2.84	-0.97	Decrease
1,66058518,1,C/T	-3.502	Deleterious	0.12	TOLERATED	2.99	-1.54	Decrease
1,66070917,1,G/C	-1.287	Neutral	0.41	TOLERATED	2.79	-0.96	Decrease
1,66067583,1,G/C	-2.707	Deleterious	0	DAMAGING	2.79	-0.8	Decrease
1,66036359,1,T/G	-1.033	Neutral	0.29	TOLERATED	2.84	-1.2	Decrease
1,66083818,1,A/C	-2.547	Deleterious	0.11	TOLERATED	2.79	-1.26	Decrease
1,66067132,1,A/G	-2.712	Deleterious	0.25	TOLERATED	2.79	-0.32	Decrease
1,66102256,1,C/G			0.38	TOLERATED	2.83	-0.43	Decrease
1,66062266,1,T/G	-0.887	Neutral	0.35	TOLERATED	2.87	-2.48	Decrease
1,66101890,1,A/G	-3.382	Deleterious	0.04	DAMAGING	2.8	0.12	Decrease
1,66101991,1,A/G	-0.142	Neutral	1	TOLERATED	2.8	-0.38	Decrease
1,66102517,1,C/T			0.06	TOLERATED	2.84	-0.74	Decrease
1,66064343,1,G/C	-1.872	Neutral	0.19	TOLERATED	2.87	-0.75	Decrease
1,66081880,1,T/G	-1.111	Neutral	0.04	DAMAGING	2.79	-1.73	Decrease
1,66083689,1,G/A	-0.961	Neutral	0.02	DAMAGING	2.79	-0.84	Decrease
1,66062274,1,G/A	-0.494	Neutral	0.58	TOLERATED	2.87	-0.79	Decrease
1,66062203,1,G/T	-2.995	Deleterious	0.06	TOLERATED	2.87	0.25	Increase
1,66102444,1,G/A	-	-	0.77	TOLERATED	2.83	-0.08	Decrease
1,66036432,1,T/A	-0.259	Neutral	0.04	DAMAGING	2.87	-2.15	Decrease

(Table 2). Continued.

Coordinates	Provean score	Provean Prediction	SIFT Score	SIFT Prediction	Median Info	SVM2 Prediction Effect (Kcal/mol)	DDG Value Prediction
1,66101944,1,C/T	-3.266	Deleterious	0.01	DAMAGING	2.8	-0.55	Decrease
1,66081785,1,A/G	-2.01	Neutral	0.15	TOLERATED	2.79	-0.16	Decrease
1,66062214,1,G/A	-0.217	Neutral	0.47	TOLERATED	2.87	-0.23	Decrease
1,66070918,1,T/A	-2.59	Deleterious	0.04	DAMAGING	2.79	-0.88	Decrease
1,66036162,1,T/C	-1.484	Neutral	0.02	DAMAGING	3.05	-2.31	Decrease
1,66102037,1,C/T	-	-	0.14	TOLERATED	3.03	-0.55	Decrease
1,66087072,1,A/C	-5.087	Deleterious	0.01	DAMAGING	2.79	-1.18	Decrease
1,66036344,1,A/G	-0.019	Neutral	0.57	TOLERATED	2.86	-1.53	Decrease
1,66036242,1,A/G	-1.272	Neutral	0.03	DAMAGING	2.99	-1.25	Decrease
1,66067538,1,A/G	-2.996	Deleterious	0.24	TOLERATED	2.79	-0.36	Decrease
1,66036256,1,C/G	-0.469	Neutral	0.28	TOLERATED	2.99	-1.36	Decrease
1,66102057,1,G/A			0.71	TOLERATED	2.83	-0.32	Decrease
1,66036381,1,G/T	-5.35	Deleterious	0	DAMAGING	2.79	-0.3	Decrease
1,66075748,1,A/G	-1.804	Neutral	0.08	TOLERATED	2.79	-0.59	Decrease
1,66075916,1,A/C	-0.08	Neutral	0.21	TOLERATED	2.79	-0.38	Decrease
1,66102312,1,A/G			0.54	TOLERATED	2.83	0.18	Increase
1,66070787,1,G/T	-1.529	Neutral	0.23	TOLERATED	2.79	-1.12	Decrease
1,66102261,1,A/G			0.32	TOLERATED	2.83	-0.08	Increase
1,66083743,1,T/A	-0.377	Neutral	0.06	TOLERATED	2.79	-1.02	Increase
1,66075778,1,T/C	-0.388	Neutral	0.23	TOLERATED	2.79	-0.78	Decrease
1,66036270,1,G/A	-1.504	Neutral	0.01	DAMAGING	3.05	-0.84	Decrease
1,66067209,1,C/T	-5.614	Deleterious	0.02	DAMAGING	2.79	-1.53	Decrease
1,66102598,1,C/A	-	-	0.01	DAMAGING	2.94	-1.09	Decrease
1,66038061,1,C/G	-1.099	Neutral	0.01	DAMAGING	2.79	-1.39	Decrease
1,66062176,1,A/G	-2.133	Neutral	0.36	TOLERATED	2.87	-1.13	Decrease
1,66101908,1,C/G	-0.873	Neutral	0.09	TOLERATED	2.8	-0.27	Decrease
1,66058435,1,T/C	-0.517	Neutral	0.33	TOLERATED	2.79	-1.19	Decrease
1,66101907,1,A/G	-0.458	Neutral	0.55	TOLERATED	2.8	-1.03	Decrease
1,66067321,1,A/G	-1.426	Neutral	0.21	TOLERATED	2.8	-0.2	Decrease
1,66102429,1,A/G	-	-	1	TOLERATED	2.83	-	-
1,66067152,1,A/G	-1.344	Neutral	0.51	TOLERATED	2.99	-0.19	Decrease
1,66081884,1,C/A	-3.772	Deleterious	0	DAMAGING	2.79	-1.11	Decrease
1,66067105,1,C/G	-3.068	Deleterious	0.02	DAMAGING	2.79	-0.6	Decrease
1,66101940,1,G/C	-0.984	Neutral	0.06	TOLERATED	2.8	-0.61	Decrease
1,66081812,1,C/T	-3.2	Deleterious	0.04	DAMAGING	2.79	0.06	Increase
1,66074556,1,G/T	-1.77	Neutral	0.06	TOLERATED	2.79	-0.57	Decrease
1,66062194,1,C/T	-	-	0.21	TOLERATED	2.87	-0.17	Decrease
1,66074478,1,T/C	-0.072	Neutral	0.67	TOLERATED	2.79	-2.11	Decrease
1,66102451,1,C/T	-	-	0.21	TOLERATED	2.83	-	-
1,66067176,1,A/G	-0.16	Neutral	0.31	TOLERATED	2.79	-1.04	Decrease
1,66038029,1,T/A	-5.004	Deleterious	0.01	DAMAGING	2.79	-0.64	Decrease

(Table 2). Continued.

Coordinates	Provean score	Provean Prediction	SIFT Score	SIFT Prediction	Median Info	SVM2 Prediction Effect (Kcal/mol)	DDG Value Prediction
1,66102542,1,A/C	-	-	0.18	TOLERATED	2.84	-1.45	Decrease
1,66087066,1,G/A	-2.278	Neutral	0.12	TOLERATED	2.83	-1.06	Decrease
1,66064359,1,C/T	-1.776	Neutral	0.1	TOLERATED	2.87	-0.18	Decrease
1,66102151,1,T/C	-	-	0.01	DAMAGING	2.83	-	-
1,66067534,1,A/G	-0.38	Neutral	0.03	DAMAGING	2.79	-1.24	Decrease
1,66064386,1,T/C	-1.846	Neutral	0.07	TOLERATED	2.87	-2.39	Decrease
1,66036251,1,T/C	-1.014	Neutral	0.49	TOLERATED	3.02	-1.45	Decrease
1,66102160,1,C/A	-	-	0	DAMAGING	2.83	-0.26	Decrease
1,66038059,1,A/G	-0.348	Neutral	0.28	TOLERATED	2.79	-0.73	Decrease
1,66062149,1,T/C	-2.106	Neutral	0.02	DAMAGING	2.87	-2.26	Decrease
1,66036362,1,T/C	-0.337	Neutral	0.22	TOLERATED	2.84	-0.44	Increase
1,66070776,1,G/A	-2.071	Neutral	0.41	TOLERATED	2.79	-1.01	Decrease
1,66067559,1,G/A	-4.29	Deleterious	0	DAMAGING	2.79	-0.9	Decrease
1,66102678,1,A/G	-1.81	Neutral	0.12	TOLERATED	3.21	-0.81	Decrease
1,66062259,1,A/G	-0.964	Neutral	0.58	TOLERATED	2.87	-1.5	Decrease
1,66036184,1,C/A	-1.099	Neutral	0.22	TOLERATED	2.99	-0.16	Decrease
1,66102061,1,G/T	-	-	0	DAMAGING	2.83	-0.13	Decrease
1,66036461,1,A/C	-0.408	Neutral	0.62	TOLERATED	2.87	-0.71	Decrease
1,66058476,1,A/G	-0.606	Neutral	0.08	TOLERATED	2.8	-0.48	Decrease
1,66036194,1,C/G	-1.234	Neutral	0.25	TOLERATED	2.99	-1.95	Decrease
1,66102078,1,A/G			0.12	TOLERATED	2.89	-1.68	Decrease
1,66064422,1,G/A	-1.097	Neutral	0.19	TOLERATED	2.87	-0.87	Decrease
1,66102171,1,A/G	-0.772	Neutral	0.15	TOLERATED	2.83	-1.12	Decrease
1,66102520,1,C/A			0.28	TOLERATED	2.84	-2.16	Decrease
1,66083760,1,A/C	-1.326	Neutral	0.16	TOLERATED	2.79	-0.26	Decrease
1,66067345,1,A/C	-4.718	Deleterious	0.01	DAMAGING	2.79	-1.2	Decrease
1,66038071,1,G/A	-0.562	Neutral	0.13	TOLERATED	2.99	-0.94	Decrease
1,66074525,1,G/C	-0.787	Neutral	0.37	TOLERATED	2.79	-0.54	Decrease
1,66075974,1,T/A	-10.01	Deleterious	0	DAMAGING	2.79	-0.84	Decrease
1,66067119,1,A/C	-3.134	Deleterious	0.01	DAMAGING	2.79	-0.72	Decrease
1,66067227,1,G/C	-1.189	Neutral	0.54	TOLERATED	2.79	-0.68	Decrease
1,66062211,1,T/A	-0.221	Neutral	0.22	TOLERATED	2.87	-1.12	Decrease
1,66102295,1,A/C			0.91	TOLERATED	2.83	-1.11	Decrease
1,66031253,1,T/C	-0.694	Neutral	0.4	TOLERATED	3.32	-1.89	Decrease
1,66058500,1,G/A	-1.761	Neutral	0.19	TOLERATED	2.79	-0.38	Decrease
1,66102447,1,A/G			0.19	TOLERATED	2.83	-1.39	Decrease
1,66102619,1,C/G			0.08	TOLERATED	2.84	-0.51	Decrease
1,66102472,1,G/C			0.16	TOLERATED	2.84	-0.87	Decrease
1,66058503,1,G/A	-0.34	Neutral	0.46	TOLERATED	2.79	-0.68	Decrease
1,66038068,1,G/A	0.052	Neutral	0.4	TOLERATED	2.79	-1.17	Decrease
1,66070798,1,G/A	-2.317	Neutral	0.13	TOLERATED	2.79	-1.01	Decrease

(Table 2). Continued.

Coordinates	Provean score	Provean Prediction	SIFT Score	SIFT Prediction	Median Info	SVM2 Prediction Effect (Kcal/mol)	DDG Value Prediction
1,66067143,1,A/G	-1.765	Neutral	0.08	TOLERATED	2.8	-0.88	Decrease
1,66058456,1,A/G	-0.736	Neutral	0.3	TOLERATED	2.79	-0.04	Decrease
1,66102679,1,T/C	-3.148	Deleterious	0.46	TOLERATED	3.21	-0.16	Decrease
1,66067110,1,G/A	-0.433	Neutral	0.33	TOLERATED	2.79	-0.78	Decrease
1,66102219,1,A/T	-1.14	Neutral	0.21	TOLERATED	2.83	-0.66	Decrease
1,66067326,1,C/T	-0.023	Neutral	0.14	TOLERATED	2.8	0.34	Increase
1,66036351,1,T/C	-0.932	Neutral	0.09	TOLERATED	2.94	-1.62	Decrease
1,66070891,1,C/T	-3.923	Deleterious	0.05	DAMAGING	2.79	-0.98	Decrease
1,66075712,1,G/A	-4.402	Deleterious	0	DAMAGING	2.79	-1.34	Decrease
1,66070728,1,C/A	-0.807	Neutral	0.27	TOLERATED	2.79	-1.04	Decrease
1,66101898,1,A/G	0.004	Neutral	0.86	TOLERATED	2.8	-0.59	Decrease
1,66064401,1,C/G	-2.91	Deleterious	0.03	DAMAGING	2.87	-0.16	Increase
1,66031287,1,G/T	-2.548	Deleterious	0.03	DAMAGING	3.06	-1.46	Decrease
1,66067188,1,A/G	-0.896	Neutral	0.1	TOLERATED	2.79	-0.9	Decrease
1,66102118,1,C/A			0.23	TOLERATED	2.83	-1.72	Decrease
1,66074545,1,G/T	-3.16	Deleterious	0	DAMAGING	2.79	-1.09	Decrease
1,66101959,1,A/G	-3.01	Deleterious	0	DAMAGING	2.8	-1.1	Decrease
1,66067613,1,C/A	-0.582	Neutral	1	TOLERATED	2.79	-0.09	Decrease
1,66102403,1,C/G			0.01	DAMAGING	2.83	-0.55	Decrease
1,66087086,1,G/A	0.326	Neutral	1	TOLERATED	2.79	-0.78	Decrease
1,66083719,1,C/A	-5.131	Deleterious	0	DAMAGING	2.79	-1.7	Decrease
1,66058348,1,T/C	-1.002	Neutral	0.31	TOLERATED	2.99	-1.72	Decrease
1,66102375,1,G/A	-	-	0.12	TOLERATED	2.83	-0.15	Decrease
1,66038009,1,A/G	0.377	Neutral	1	TOLERATED	2.87	-0.76	Increase
1,66081791,1,C/G	-3.278	Deleterious	0	DAMAGING	2.79	-0.11	Increase
1,66070824,1,A/G	-0.66	Neutral	0.25	TOLERATED	2.79	-0.66	Decrease
1,66074533,1,C/G	-1.512	Neutral	0.16	TOLERATED	2.79	-0.31	Decrease
1,66075952,1,G/C	-0.068	Neutral	0.16	TOLERATED	2.79	-0.46	Decrease
1,66058513,1,A/G	-1.271	Neutral	0.39	TOLERATED	2.99	-0.18	Decrease
1,66036441,1,A/G	-0.378	Neutral	0.51	TOLERATED	2.87	-0.5	Decrease
1,66036368,1,A/G	-0.576	Neutral	0.3	TOLERATED	2.79	-1.35	Decrease

interaction suggests functional relevance, with a PPI enrichment p-value of 0.0583. While not statistically significant, this enrichment value hints at a meaningful biological association between leptin and *LEPR* that aligns with their established role in metabolic signalling pathways.

STRING analysis of the interaction between the leptin gene and leptin receptor (leptin R).

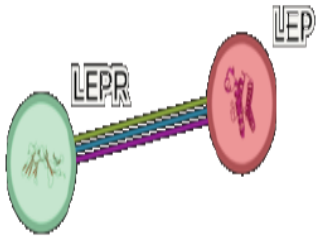

Figure 2: Protein -protein interactions of leptin & leptin receptor.

Table 3: Gene Ontology Enrichment -Biological Process

Description	Count in network	Strength	Signal	False discovery rate
Leptin-mediated signaling pathway	2 of 11	3.25	2.15	0.0063
Bone growth	2 of 30	2.82	1.93	0.01
Regulation of bone remodeling	2 of 50	2.6	1.6	0.0214
Regulation of gluconeogenesis	2 of 51	2.59	1.6	0.0214
Energy reserve metabolic process	2 of 66	2.47	1.5	0.0263
Negative regulation of autophagy	2 of 87	2.35	1.37	0.0352
Positive regulation of cold-induced thermogenesis	2 of 97	2.31	1.32	0.0392
Cholesterol metabolic process	2 of 119	2.22	1.3	0.0398
Glucose metabolic process	2 of 116	2.23	1.3	0.0398
Carbohydrate biosynthetic process	2 of 133	2.17	1.26	0.043

Table 4: KEGG Pathway

pathway	description	count in network	strength	signal	false discovery rate
hsa04920	Adipocytokine signaling pathway	2 of 68	2.46	2.21	0.0042
hsa04152	AMPK signaling pathway	2 of 120	2.22	1.97	0.0064
hsa04932	Non-alcoholic fatty liver disease	2 of 146	2.13	1.93	0.0064
hsa04630	JAK-STAT signaling pathway	2 of 158	2.1	1.91	0.0064
hsa04060	Cytokine-cytokine receptor interaction	2 of 282	1.84	1.53	0.0139
hsa04080	Neuroactive ligand-receptor interaction	2 of 329	1.78	1.45	0.0158

The interaction network between *LEP* and *LEPR*, generated using STRING analysis, shows a significant association with a p-value of < 0.05. The results highlight the critical roles of leptin and its receptor in key biological processes and signalling pathways. Gene ontology enrichment analysis identified the leptin-mediated signalling pathway, bone growth, and regulation of gluconeogenesis as significantly enriched processes, reflecting leptin's involvement in energy homeostasis, metabolic regulation, and skeletal health (Table **3**). Metabolic processes such as glucose metabolism, cholesterol metabolism, and energy reserve management were also prominent, emphasizing leptin's central role in systemic metabolic balance. KEGG pathway analysis further revealed leptin's integration into critical signalling pathways, including the adipocytokine and AMPK pathways, which regulate energy expenditure and insulin sensitivity (Table **4**). Additionally, leptin's involvement in immune and neuroendocrine signalling was underscored by its enrichment in the JAK-STAT, cytokine-cytokine receptor interaction, and neuroactive ligand-receptor interaction pathways, highlighting its dual role in metabolic regulation and inflammatory processes. These findings underscore leptin's multifaceted functions in metabolic and physiological regulation.

DISCUSSION

This study demonstrates that *LEP* and *LEPR* SNPs significantly influence weight loss, metabolic outcomes, and hormonal responses after bariatric surgery. The bioinformatics analyses provided mechanistic insights into these associations, emphasizing leptin's central role in energy metabolism, appetite regulation, and inflammatory processes.

Patients carrying risk alleles (e.g., *LEP* rs7799039 G and *LEPR* rs1137101 G) exhibited diminished postoperative improvements, likely due to impaired leptin signalling and sensitivity. Pathway enrichment and PPI analyses further supported leptin's involvement in neuropeptide signalling and adipogenesis, underscoring its dual role in weight regulation and systemic inflammation.

These findings highlight the value of integrating genetic data with bariatric care. Identifying at-risk genotypes could enable tailored preoperative counselling and targeted postoperative interventions, improving long-term outcomes.

Protein structure, stability, and subsequently function is affected by mutations. The "raw material" of evolution includes mutations. On the other side, negative, purifying selection eliminates the majority, if not all, protein mutations, reducing the likelihood of future adaptations. Because of this, under the influence of positive selection, only a small portion of all potential mutations will be fixed to take on a new function. Due to randomness, or "neutral drift," neutral mutations can potentially stochastically fix in small populations. Mutations' effects on fitness at the organismal level are complicated, and they infrequently correlate with the characteristics of a single gene or protein. Redundancy, backup, and resilience at several levels mitigate the effects of numerous mutations [16]. Indeed, understanding and predicting the effects of mutations on the organismal level is a major challenge of evolutionary biology [17,18].

The amount of functional protein present affects the stability of proteins. An investigation of pathogenic mutations revealed that stability and folding effects account for 80% of the detrimental consequences of pathogenic mutations [19]. Mutations that are destabilising above a specific threshold (or DDG value) by reducing the quantities of soluble, function proteins are the source of protein defunctionalisation [19]. The likelihood of a deleterious mutation is in the range of 33-40 percent, according to experimental data in a variety of proteins [18] (On average, 36 percent). Protein fitness thus declines dramatically as mutations mount. A protein's fitness is reduced to 20% after five mutations have been added to it.

Although a protein's initial stability can mitigate some of the destabilising effects of mutations, stability seems to be the primary (though undoubtedly not the only) factor that governs how quickly proteins evolve, and perhaps even how quickly entire organisms do as well [20,21], particularly but not exclusively in relation to the acquisition of new functions.

Experimental datasets are often provided for a small subset of proteins and are typically related to changes in mutation thermodynamic stability (DDG values). Recent advances in computation now allow us to anticipate the DDG values of mutations in a wide range of proteins. Sequence is a key component of some prediction methods [22], while three-dimensional structures are a key component of others [43].

Predictions exclude effects on folding intermediates and largely focus on how mutations affect the native state. Forecasts of kinetic stability effects would be very helpful even though they may overlap with thermodynamic stability effects *in vivo*. Overall, further research is needed to provide more accurate and realistic estimations of how mutations affect protein levels *in vivo* [24].

It appears that minor kcal/mol stability losses lead to a significant drop in protein levels by producing a large enough fraction of partially folded and/or misfolded species to cause irreversible aggregation or degradation.

Stability decreases beyond the permitted margin as more mutations are added, leading to fitness loss along with DG changes.

The destabilising effects of mutations prevent the creation of novel protein functions. On the other hand, it has been noted that neutral or non-adaptive mutational drifts are less disruptive and tend to occur at more buried residues than new function or adaptive mutations [25].

Regardless of whether SIFT and Provean analyses of SNPs in the leptin and leptin receptor genes suggest that they are harmful or tolerable, I mutant analysis demonstrates the lower thermodynamic stability of the proteins. The altered function of leptin and leptin receptor proteins may result from this. This result lends credence to a study on leptin and leptin gene polymorphisms in obese people and their susceptibility to depression.

Although there are already a number of studies demonstrating the relationship between SNPs in various genes and various disorders, computational investigation of the functional effects of SNPs in this gene has not yet been done. The SIFT technique uses sequence homology among related genes and domains over evolutionary time, as well as the physical-chemical properties of the amino acid residues, to predict whether an amino acid change would affect protein function. The "false negative" and "false positive" error rates of SIFT are estimated to be 31% and 20%, respectively. SIFT is about 80% successful in benchmarking studies using amino acid substitutions assumed to have a significant negative

impact on the residual activity of the variant protein as the test set. However, SIFT and provean can be very helpful in predicting how a mutation will affect how a protein functions as well as the necessity of evaluating gene polymorphisms using wet lab techniques. I mutant evaluated the stability of the mutant proteins because the majority of disease mutations have an impact on protein stability.

Similar *In silico* analysis was carried out by Dakal *et al.* who explored identification, characterization and validation of deleterious non-synonymous SNPs (nsSNPs) in the interleukin-8 gene for predicting its functional consequences [26].

Leptin (*LEP*) is a hormone specifically produced by adipocytes, and its serum concentration is proportional to body fat mass which, in turn, has its amount regulated by the hypothalamic effects of *LEP* gene. Intravenous administration of *LEP* reduces appetite; while its deficiency increases food intake [27]. Its action occurs through the leptin receptor (*LEPR*), which is encoded by the *LEPR* gene. *LEPR* is a single-transmembrane-domain receptor of the cytokine-receptor family with widespread tissue distribution and several alternatively spliced isoforms [28].

Several *LEPR* mutations have been described in patients with early-onset of severe obesity and hyperphagic eating behaviour [29,30]. In contrast, a protective influence of two polymorphisms (rs1137100 and rs1137101) to higher blood pressure levels in men has been identified, increasing the protection when the carriers have the arginine allele in the two single nucleotide polymorphisms (SNPs) [31].

The gene ontology (GO) enrichment analysis revealed key biological processes associated with leptin and its receptor, underscoring their central roles in metabolic regulation, bone health, and energy homeostasis (Table **3**). Among the enriched processes, the leptin-mediated signalling pathway showed the strongest enrichment (strength = 3.25, false discovery rate (FDR) = 0.0063), reflecting leptin's critical function in appetite regulation, energy expenditure, and hormonal signalling. This pathway directly connects leptin to downstream molecular cascades that influence metabolic and physiological outcomes.

Several other enriched processes highlight leptin's involvement in skeletal and metabolic regulation. Bone growth (strength = 2.82, FDR = 0.01) and regulation of bone remodelling (strength = 2.6, FDR = 0.0214) suggest that leptin may play a role in bone development and the balance between bone resorption and formation. This aligns with leptin's known influence on bone metabolism via its interaction with hypothalamic and peripheral pathways.

Metabolic processes were also prominently enriched, with regulation of gluconeogenesis (strength = 2.59, FDR = 0.0214) and energy reserve metabolic processes (strength = 2.47, FDR = 0.0263) reflecting leptin's critical role in glucose homeostasis and energy storage. Leptin's regulatory impact on glucose metabolism, highlighted by the enrichment of glucose metabolic process (strength = 2.23, FDR = 0.0398), emphasizes its importance in maintaining systemic metabolic balance, particularly relevant to obesity and related disorders.

Additionally, pathways like negative regulation of autophagy (strength = 2.35, FDR = 0.0352) and positive regulation of cold-induced thermogenesis (strength = 2.31, FDR = 0.0392) underscore leptin's role in adaptive responses to energy demands and environmental changes. Processes such as cholesterol metabolic process (strength = 2.22, FDR = 0.0398) and carbohydrate biosynthetic process (strength = 2.17, FDR = 0.043) further support leptin's multifaceted role in lipid and carbohydrate metabolism.

The KEGG pathway analysis provided further insights into the functional roles of leptin and its receptor within broader signalling networks (Table **4**). The adipocytokine signalling pathway (strength = 2.46, FDR = 0.0042) emerged as the most enriched pathway, consistent with leptin's role as a key adipocyte-derived hormone regulating energy balance, inflammation, and insulin sensitivity. This pathway integrates leptin into a network of adipocytokines influencing metabolic homeostasis.

The AMPK signalling pathway (strength = 2.22, FDR = 0.0064) highlights leptin's interaction with AMP-activated protein kinase, a master regulator of energy balance. This pathway underscores leptin's role in promoting energy expenditure and maintaining glucose and lipid homeostasis, particularly under conditions of metabolic stress.

Enrichment in the non-alcoholic fatty liver disease (NAFLD) pathway (strength = 2.13, FDR = 0.0064) points to leptin's relevance in hepatic lipid metabolism and the prevention of fat accumulation in the liver, a common condition associated with obesity. Similarly, the JAK-STAT signalling pathway (strength = 2.1, FDR = 0.0064) reflects leptin's canonical signalling

mechanism, which is central to its effects on inflammation and metabolic regulation.

Leptin's broader role in immune and neuroendocrine signalling is highlighted by the enrichment of the cytokine-cytokine receptor interaction (strength = 1.84, FDR = 0.0139) and neuroactive ligand-receptor interaction (strength = 1.78, FDR = 0.0158) pathways. These results illustrate leptin's dual functionality in mediating immune responses and influencing neuroendocrine processes that regulate energy homeostasis and behaviour.

These enrichment analyses confirm leptin's multifaceted roles in critical biological processes and signalling pathways. From metabolic regulation and bone health to immune and neuroendocrine signalling, leptin and its receptor are integral to maintaining physiological balance, particularly in the context of obesity and metabolic disorders. These findings provide a strong foundation for further exploring leptin's therapeutic potential in metabolic and inflammatory conditions.

Leptin-related pathways are intricately linked to bariatric surgery outcomes, including weight loss, metabolic improvements, and hormonal regulation. The leptin-mediated signalling and AMPK pathways regulate appetite, energy expenditure, and glucose metabolism, directly influencing weight loss and glycaemic control after surgery. Enrichment in pathways like gluconeogenesis regulation and cholesterol metabolism underscores leptin's role in improving metabolic parameters such as insulin sensitivity and lipid profiles. Leptin's involvement in the JAK-STAT and cytokine-cytokine receptor interaction pathways highlights its role in modulating immune and inflammatory responses, which are crucial for recovery and long-term health after surgery. Additionally, leptin's participation in neuroactive ligand-receptor interactions ties it to hormonal and neuroendocrine regulation, affecting appetite control and mood. The regulation of bone growth and remodelling pathways further connects leptin to skeletal health, often impacted by post-surgical changes. Together, these pathways reveal leptin's central role in mediating the physiological and metabolic benefits of bariatric surgery and highlight how genetic variations in leptin signalling may influence individual outcomes.

The bioinformatics findings underscore the possibility for incorporating genetic data into clinical procedures, facilitating more accurate predictions of patient outcomes post-bariatric surgery from a statistical modelling viewpoint. Clinicians might customize preoperative counselling and postoperative therapies by integrating SNP-based risk assessments. It is crucial to acknowledge potential biases in SNP selection, since an emphasis on certain variations may neglect other pertinent genetic features. Moreover, assumptions inherent in statistical models, such as the independence of SNP effects or homogeneity across populations, may influence the generalizability of results.

CONCLUSION

Bioinformatics analyses of leptin gene polymorphisms reveal their critical influence on bariatric surgery outcomes. SNPs of leptin and leptin receptor modulate weight loss, metabolic changes, and hormonal responses, offering new avenues for personalized obesity management. These pathways collectively highlight how leptin and its receptor influence the multifaceted outcomes of bariatric surgery. Genetic variations in leptin signalling can impact the degree of weight loss, metabolic improvements, and hormonal adaptations, making leptin-related pathways critical to understanding individual variability in surgical success. Exploring these connections further could pave the way for personalized interventions and targeted therapies to optimize bariatric surgery outcomes. The integration of bioinformatics with sophisticated statistical methodologies presents a robust strategy for future investigations in precision medicine. Researchers can uncover significant SNPs and pathways that affect individual responses to bariatric surgery by integrating genetic data with statistical modelling, so facilitating more accurate predictions and enhancements of treatment results. This method facilitates the identification of fundamental biological mechanisms and enhances the formulation of individualized treatments, enabling the customization of medicines according to genetic profiles and augmenting the overall efficacy of surgical procedures.

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CONFLICTS OF INTEREST

None.

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