Journal of Positive School Psychology http://journalppw.com
2022, Vol. 6, No. 7, 3681-3706

In Silica Analysis Of Single Nucleotide Polymorphisms Of Leptin And Leptin Receptor Gene : A Preliminary Bioinformatics Data

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Abstract

Introduction: Depression and obesity are two common disorders that regularly co-occur in people and have substantial public health repercussions. Both conditions are linked in a bidirectional way: having one increases the chances of obtaining the other. Leptin is the adipokine hormone that has a role in obesity as well as known to influence the mood. Mutations of leptin and its receptor genes have been least studied in both the conditions. The aim of the study is to carry out the in silica analysis of leptin and its receptor gene using bioinformatics tools to predict the functional effects of the non-synonymous SNPs of these genes.

Method: Insilico analysis of SNPs of leptin and its receptor gene were carried out using their accession IDs and their FASTA amino acid sequences obtained from NCBI. SIFT(Sorting the intolerant from tolerant), Provean (protein variation effect analyzer) and I mutant 3.0 were the bioinformatics tools used for the analysis.

Results: Analysis ofSNPs ofleptin gene by SIFT revealed 75% tolerated and 25% damaging mutations. Provean analysis showed 31% deleterious and 69% neutral mutations. On I mutant analysis,85% of the SNPs resulted in decreased thermodynamic stability whereas 15% of them had increased stability. 69% of SNPs of leptin receptor genes were found to be damaging on SIFT,18% on Provean and 94.4% of them showed decreased stability.

Conclusion: The study suggests strongly that deleterious effects of mutations on leptin and its receptor as well as their reduced stability predicted by the bioinformatics toolsaffect their structure and function. Mutations of leptin receptor may be more deleterious compared to that of leptin gene.These mutations may influence the pathobiology of depression as well asobesityand wet lab study on these genes may be useful in linking the pathogenesis of obesity and depression.

Key words: leptin,receptor, gene polymorphisms, bioinformatics

I. BACKGROUND

Depression and obesity are two common illnesses with serious public health consequences that frequently co-occur in people. The link between both conditions is bidirectional: having one raises your chances of getting the other. As a result, gaining a deeper understanding of the mechanisms underlying the interwoven downward physiological spirals associated with both illnesses has become critical. Genetics, alterations in systems involved in homeostatic adjustments such as the hypothalamus pituitary axis, immuno-inflammatory activation, neuroendocrine regulators of energy metabolism such as leptin and insulin, microbiome, and brain circuitries integrating homeostatic and mood regulatory responses are some of the shared biological pathways that may mechanistically explain the depression–obesity link. These biological pathways may act in two, non-mutually exclusive, ways: as common underlying mechanisms influencing the liability to both depression and obesity, or as mediating mechanisms in causal relationships between the two conditions.

The study aims to explore one of the important biological links between depression and obesity and neuroendocrine regulatory factors, which may be of great value in predicting depression in obese individuals as well as in planning therapeutic interventions in obese individuals with depression.

Leptin & obesity

The leptin–melanocortin pathway is a key neuroendocrine regulator of energy homeostasis. Leptin is produced by white adipose tissue in proportion to body fat and acts as an adiposity negative signal. When leptin binds to receptors in the hypothalamus, pro-opio melanocortin neurons are activated, and they interact with other brain centres to integrate physiological and behavioural processes that inhibit food intake and promote energy expenditure [1]. Rare extreme types of obesity, characterised by severe hyperphagia, are caused by loss-of-function mutations in critical genes in the system [2]. Obesity is related with leptin resistance, which blunts the anorexigenic impact of the hormone and, as a result, disinhibits appetite despite elevated circulating leptin levels. Central resistance is due to impaired leptin transport across the blood–brain barrier, reduced function of leptin receptors, and defects in leptin signal transduction [3].

Leptin & depression

It's also been proposed that leptin has an effect on mood. Peripheral and central administration of leptin has antidepressant-like effects in behavioural tests and reverses depressive-like behaviour generated by chronic unpredictable stress in animal models [4,5]. Direct impact on neurons via receptors expressed in the hippocampus and amygdala, increase of neurogenesis and neuroplasticity in the hippocampus and cortex, and manipulation of the HPA axis and immune system are all possible mechanisms for leptin's effects on mood [6]. Leptin resistance (peripheral hyperleptinemia due to diminished central signalling) has been proposed as a phenotypic risk factor for depression [7].

Hyperleptinemia with depression-like symptoms is consistently caused by genetic deletions of leptin receptor in the hippocampus and brain of mice [8] and resistance to treatment with fluoxetine and desipramine [9]. Independent of BMI, major depressive disorder (MDD) patients with elevated neuro vegetative symptoms (in particular hunger and weight) had more circulating leptin than healthy controls, according to a study [10] with a large sample size. Furthermore, higher leptin levels were linked to hyperphagia and weight gain in current MDD patients, independent of BMI. A study found that MDD patients had elevated appetite/weight symptoms, which were connected with polygenic risk scores for circulating leptin[11].

The volume of information on the involvement of leptin signaling in depression in humans is minimal and contentious. According to one investigation, there was no difference in leptin

levels between depressive patients and healthy controls [12]. Plasma leptin levels were observed to be greater in depressive individuals in two investigations [13], with a gender bias. Low leptin levels, on the other hand, have been linked to depression in other studies. Two studies reported that plasma leptin levels were lower in individuals with serious depression, regardless of body fat index [14,15]. In addition, suicide attempters with depression had lower levels of leptin in their cerebrospinal fluid than those without depression [16,17].

Furthermore, patients with bipolar disorder[18] and obsessive-compulsive disorder with concomitant severe depression [19] have lower levels of leptin in their plasma. These clinical findings imply a relationship between low leptin levels and serious depression when taken together. One explanation for the seemingly contradicting evidence is that leptin levels are regulated by a variety of parameters including age, gender, sample size, body mass index, and comorbidity with other illnesses. Another theory is that leptin deficiency only affects a small percentage of depressive patients. While clinical trials into leptin's antidepressant efficacy in humans are still underway, it is thought that depressed patients with low leptin levels may have a better probability of responding to leptin therapy.

The link between obesity and depression is an intriguing subject that has yet to be answered. In contrast to the concept that leptin deficiency causes depression, epidemiologic and clinical research reveal a relationship between obesity and depression [20]. Obesity is frequently characterised by high, not low, levels of leptin. Obese people are 20 percent more likely than non-obese people to experience depressive disorders, according to research [20].

Leptin resistance, similar to insulin resistance in type 2 diabetes patients, is hypothesised to be the source of high leptin levels associated with obesity. In obese adults, leptin treatment is inefficient at reducing food intake and boosting energy expenditure, whereas leptin administration causes a reduction in adipose tissue and weight loss in people of normal weight [21]. Leptin resistance is known to be induced by deficiencies in the leptin signalling system on numerous levels, including poor leptin transport across the blood-brain barrier, diminished leptin receptor activity, and defects in leptin signal transduction [22].

It's possible that leptin resistance contributes to the greater rate of depression in obese persons, given leptin's capacity to prevent depressive behaviours in animal models. This could also aid in the interpretation of some of the contradictory findings on circulating leptin levels in depressed patients. One important topic is whether leptin resistance is a shared biological component in the obesity-depression comorbidity. Therapeutic approaches that target leptin downstream pathways and overcome leptin resistance, rather than leptin itself, are expected to be more effective for obese persons with depression.

Depression and obesity are intimately linked and interact, resulting in a negative cycle in a person's health. This finding has significant clinical consequences. On the one hand, this co-occurrence could pose a significant challenge in treating each ailment independently. Obesity-related biochemical dysregulations have been linked to a more chronic course in depressive individuals [23] and a poor response to traditional antidepressant therapies [24]. Similarly, comorbid depression may impair adherence to obesity and related-conditions treatments by reducing adherence to medication and lifestyle recommendations. On the other hand, this association could be useful in treating people who have comorbid depression and obesity.

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 3-billion-base-long human genome contains SNPs at intervals of 100–300 bases, with varying density in various regions [25]. 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 [26].Coding synonymous SNPs (sSNPs), as well as non-coding SNPs (sSNPs), can nevertheless have an impact on transcription factor binding, splicing, and gene expression [27,28].

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 [29]. 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 [30,31].

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 [29].

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 [30]. 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.

It may be possible to establish a cause-and-effect relationship between obesity and depression through conducting anoriginal research by using in silico investigation results of leptinandleptin receptors. The proposed research aims to investigate all missense-mutated single nucleotide polymorphisms (SNPs) of leptin and leptin receptor and find out the deleterious ones. The sole purpose of these bioinformatics tools is to rank SNPs according to their functional importance.

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 silica analysis of leptin and its receptor gene using bioinformatics tools such as sorting the intolerant from tolerant(SIFT), Provean and I-mutantsoftwares.This study may emphasize on the necessity to conduct an experimental study to explore the possible influence of mutations of these genes in the pathogenesis of depression and obesity.

II. METHODOLOGY

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

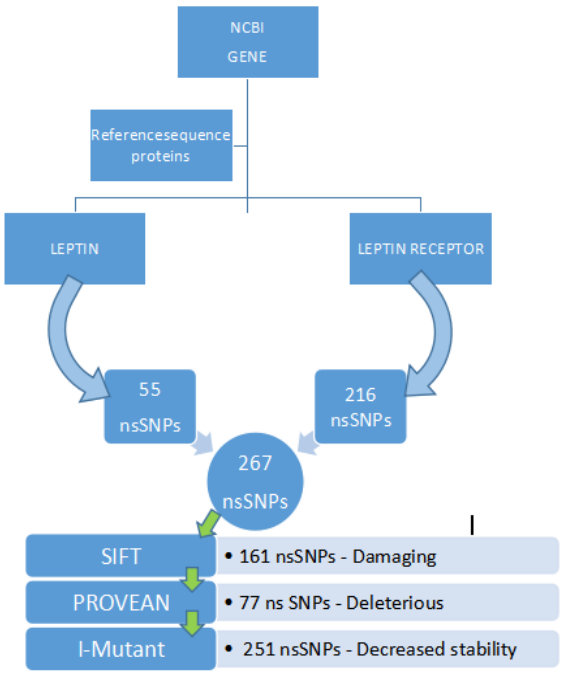

Fig 1: *Depicting the analysis of genes using bioinformatics tools*

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 [32]. 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[33].

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 nsSNPsUsing 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[34]. 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 [34,35]. 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 [36,37] 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 maximisesensitivity and specificity for determining which human

protein variations commonly cause disease and which have functional effects [38].

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.

III.RESULTS AND DISCUSSION

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 represented at that position and this column indicates this fact.

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.

Table 1:*Analysis of SNPs of leptin gene with bioinformatics tools*

Cooridnates	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	**-0.80**	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
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	**0.00**	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	**-0.90**	Decrease
7,127894640,1,G/A	0.914	Neutral	0.03	DAMAGING	2.9	**-0.89**	Decrease

SIFT Analysis of LEPTIN Receptor 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.OnProvean 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.

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	**-0.10**	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	**-1.20**	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
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	**-1.00**	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	**-0.50**	Decrease
1,66081883, 1,A/G	-3.478	Deleterious	0	DAMAGING	2.79	**-1.50**	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
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	**-1.30**	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	**-0.80**	Decrease
1,66036359, 1,T/G	-1.033	Neutral	0.29	TOLERATED	2.84	**-1.20**	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
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	**-0.30**	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	**-0.20**	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	**-0.60**	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

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	**-0.90**	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	**-1.50**	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	**-1.20**	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
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	**-0.90**	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	**-1.10**	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	**-1.70**	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	**-0.50**	Decrease
1,66036368, 1,A/G	-0.576	Neutral	0.3	TOLERATED	2.79	**-1.35**	Decrease

Protein structure, stability, and subsequently function are 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 [39].Indeed, understanding and predicting the effects of mutations on the organismal level is a major challenge of evolutionary biology [40,41]. 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 [42]. Mutations that are destabilising above a specific threshold (or DDG value) by reducing the quantities of soluble, function proteins are the source of protein dysfunctionalization [42]. The likelihood of a deleterious mutation is in the range of 33-40 percent, according to experimental data in a variety of proteins [41]. (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 [43, 44], 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 [45], while three-dimensional structures are a key component of others [46].

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 [47].

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 [48].

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 it's functional consequences[49].

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 [50]. 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 [51].

Several LEPR mutations have been described in patients with early-onset of severe obesity and hyperphagic eating behaviour [52,53]. 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) [54].

Leptin may operate as an antidepressant, according to data from animal research, however there is currently scant support for a causal link between depression and leptin deficiency. [55,56] Despite the fact that low levels of leptin have been linked to depressed behavior's in both rodents and humans[56] research on the function of leptin signaling in human depression is scarce and, as of yet, contentious. According to Deuschle et al. there is no difference in leptin levels between depressive patients and healthy controls[57]. With a bias toward female participants, two further investigations discovered greater plasma leptin levels in depressive patients.[58,59]

It has been shown that serious depressive individuals' plasma leptin levels indeed fall, regardless of their body mass index, in a larger sample size (BMI) [60-62]. Patients with bipolar disorder have also been found to have lower plasma levels of leptin [62]. The idea that genetic variations near the LEP gene may be causally linked not only in human obesity but also connected with behavioral disorders was initially

put forth by Comings et al [63]. A functionally flawed leptin protein or changed expression brought on by changes in the LEP gene promoter can also induce altered leptin levels [64].

The relationship between depression and BMI can be understood by the fact that sad people either lose or gain weight due to a decrease in physical activity or a gain or loss of appetite, all of which are recognised symptoms of depression [65]. This may possibly be due to a shared genetic component that contributes to the development of both obesity and depression. According to Comings et al.'s analysis of the D7S1875 polymorphism, there is a direct correlation between homozygosity for the short allele (o208 bp) of the D7S1875 marker in the LEP gene and BMI[63].

There is little consensus on the subject of leptin signalling's function in human depression. According to one investigation, there was no difference in leptin levels between depressive patients and healthy controls [66]. Plasma leptin levels were found to be greater in depressive individuals in two investigations, with a bias toward female patients. [67,68].

In contrast, several studies discovered a connection between depression and low leptin levels. Two studies showed that plasma leptin levels were lowered in patients with significant depression regardless of body mass status, and these studies used larger sample sizes [69,70]. Additionally, suicidal attempters with depression had lower levels of leptin in their cerebrospinal fluid than those without depression [71,72]. Additionally, patients with bipolar illness [73] and obsessive-compulsive disorder with concurrent significant depression [74] were shown to have lower amounts of leptin in their plasma. Together, these clinical findings point to a connection between serious depression and low leptin levels.

The fact that leptin levels are impacted by variables like age, sex, sample sizes, body mass status, and comorbidity with other illnesses may be one explanation for the seemingly contradicting evidence.

The relationship between fat and depression is an intriguing unanswered subject. Contrary to the aforementioned theory that leptin deficiency fuels depression, epidemiologic and clinical data point to a connection between obesity and depression [75-78], which is frequently characterised by high, not low, levels of leptin. According to research, obese people have a roughly 20% higher chance of developing depressive disorders than non-obese ones [77].Similar to how type 2 diabetic individuals are resistant to insulin, leptin resistance is assumed to be the root cause of the elevated leptin levels linked with obesity. In fact, administering leptin to obese individuals has little effect on reducing food consumption or raising energy expenditure, whereas doing so to individuals with normal weight results in decreased adipose tissue and weight loss [79]. It is well known that leptin resistance is brought on by flaws in the leptin signalling pathway, probably at several levels, including diminished activity of the leptin receptor, poor transport of leptin across the blood-brain barrier, and flaws in leptin signal transduction [80].

There is a limited literature on the leptin and it’s receptor gene polymorphisms in obesity and depression, however altered leptin levels suggest an altered expression of these genes.

IV. CONCLUSION

The present INsilica analysis, that is designed to explore the functional and structural effects of mutations of leptin and leptin receptors by using bioinformatics tools is successful in establishing the fact that these polymorphisms are worth studying in conditions like depression, obesity. However the pathway leptin-leptin receptor gene polymorphisms-their expression that link pathobiologies of obesity and depression has not been studied so far. If such study is conducted,it may open up new molecular targets as well as biomarkers for predicting depression in obese individuals. It suggests strongly that deleterious effects of mutations on leptin and its receptor as well as their reduced stability as predicted by the bioinformatics tools may influence the pathobiology of conditions like depressionandobesity. Mutations ofleptin receptor may be more deleterious compared to that ofleptin geneand wet study on these genes

may be useful in linking the pathogenesis of obesity and depression.

Funding:
Nil

Conflicts of interest:
None

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