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Prediction of the Impact of Non-synonymous Mutation on Leptin-Ghrelin Interface

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Abstract—*A single nucleotide polymorphism can have a damaging effect on the function of a protein. Single amino acid substitutions are the result of single nucleotide variations that occurs in the gene encoding that protein. Predicting the functional effect of single amino acid substitution and linking those effects to a particular SNP could be both laborious and expensive. Bioinformatic tools offer a great deed of help in predicting the damaging effect of nsSNP and helps suggesting the SNP which will have the most deleterious effects.*
In this work we have analyzed the genetic variations that alter the function of leptin and gherlin using computational tools. Of 57 nsSNP's scrutinized in leptin, only 5 nsSNP's were found deleterious by SIFT and 2 were found deleterious by provean. Polyphen server suggested 2 would be probably damaging. Of 73 nsSNP's scrutinized in gherlin, only 3 nsSNP's were found deleterious by SIFT and 2 were found deleterious by provean. Polyphen server suggested 3 would be probably damaging. These results were further supported by other tools including PHD-SNP, nsSNP Analyzer, iMutant, and Mutation assessor. It was found by combining the results of all the software that a mutation from arginine (R) to tryptophan (W) at position 105 of the leptin peptide and a mutation of arginine (R) to glutamine (Q) at position 50 of gherlin peptide was the most deleterious. Further effects of these mutations were studied by SNPeffect and MutPred tools. Based on these results, we propose that the nsSNP with dbSNP rsid rs104894023 and rs34911341 are important candidates for the cause of diseases and disorders associated with dysfunctioning of leptin and gherlin.

Keywords*: Leptin, Ghrelin, Mutation, nsSNP, Computational tools*

1. INTRODUCTION

Obesity is one of the major consequences of lifestyle related disorders that the world is facing. As by a report of WHO, India will be the third most obese country in the world with about around 100 million obese people till 2020 (1). Body weight is balanced by a very complex system having both peripheral, central systems and environmental factors. On molecular and endocrinological level, two important factors leptin and ghrelin are crucial in body weight control and energy balance. Both are the components of peripheral system and connect to central system via different pathways. Thus genetic dispositions towards obesity could be assessed by analyzing leptin and ghrelin.

Leptin also called satiety or starvation hormone mainly secreted by adipocytes. It was the first adipokine reported (2). In 1950s the obese (ob) gene were identified in mouse and later on in 1994 identifies in human homologue by positional cloning , named as Leptin (greek "lepto" which means thin)(3,4,5). Leptin gene is present on chromosome 7q32.1, also Green et. al. mapped it on 7q31.3 (6). Leptin is composed of 3 exons and 2 introns encompassing 18kb (7). Leptin is a 16kD protein consists of 167 amino acids. It is majorly produced by adipocytes however, small amounts of its also released by different tissues such as stomach, mammary epithelium, placenta and heart (8)(9)(10). Its major functions are to restrict food intake and maintain a balance of energy expenditure, in addition to that it has variety of functions including the regulation of haematopoiesis, angiogenesis, immune response and the inflammatory response. Leptin acts through the leptin receptor (LEPR) or OBR, a single transmembrane-domain receptor of cytokine receptor family. LEPR gene mapped to chromosome 1q31 is made up of 18 exons and 17 introns, encodes protein of 1162 amino acid(11). LEPR in various spliced forms expressed in many tissues including placenta and stomach**.** Its spliced form of longest intracellular domain (OB-Rb) is widely expressed in hypothalamus and cerebellum (12). Leptin released into the circulatory system by adipose tissue, crosses blood-brain barrier, signals brain about energy store in body which leads to decrease in food intake and increase in energy expenditure to balance the body weight(13)(14).

Ghrelin or lenomorelin also called hunger hormone is a peptide hormone produced by ghrenilergic cells in stomach (15) which secrets a preprohormone called preproghrelin, which on the other hand also generates a second peptide hormone called obestatin; that acts as a ligand for GPR39 and is involved in satiety and decreased food intake (16). Gene encoding ghrelin is mapped to chromosome 3p25-p26 and consist of 4 exons and 3 introns and is of 5kb (17). Preproghrelin is 117 amino acids long which is cleaved into 28 amino acid long mature ghrelin (unacylated) and C-ghrelin (acylated). Ghrelin is made functional by ghrelin O-acyltransferase (GOAT) which adds octanoic acid chain ito

serine at 3rd position. Obestatin is thought to be derived from C-ghrelin (18). Ghrelin is produced by ghrenilergic cells in stomach mainly but also in deudonum, jejunum, lungs, gonads, pancreatic islets, adrenal cortex, placenta, kiDNAy and recently by neurons too(19). Ghrelin acts as a ligand for ghrelin hormone secretagogue receptor (GHSR). There are two types of cDNA found which encodes for GHSR referred as GHSR1a and GHSR1b. GHSR1a gene is located on chromosome 3q26 and encoded protein is of 366 amino acids, while GHSR1b is 289 amino acids long(20). When the stomach is empty, it secrets ghrelin which enters circulatory system, crosses blood brain barrier and signals the hypothalamic cells to increase hunger and increase gastric acid secretion. This also prepares the body as gastrointestinal motility for food intake. After when the stomach gets stretched, the secretion stops.

Leptin has various functions but the main function is to signal the brain to inhibit the intake of food and energy balance. Studies in rodents suggested that hypothalamus is centre for controlling of food intake and weight balance. After secretion of leptin, it binds to leptin receptors on hypothalamus cells and influences the activity of hypothalamic neurons, orexigenic and anorexigenic neuropeptides. Additionally, regulation of the effects of ghrelin on hypothalamic neurones (ghrelin prevents leptin's action by the activation of the hypothalamic NPY/Y1 receptor pathway) has been proposed to one of the principle process through leptin may control food intake and body weight (21). Leptin is also produced by stomach in small amount, hence it is said that it might play role in regulation of meal size in accordance with other satiety peptides. This gastric leptin is influenced by insulin released in bloodstream shortly after intake of food and might locally stimulate the digestion and absorption of food in intestines (22)(23). The role of ghrelin in energy maintenance is largely regulated by hypothalamus. Korbonits et al. suggested three different pathways for this, firstly circulatory ghrelin binds to its receptors on hypothalamus cells by crossing blood brain barrier. Second, ghrelin may reach via vagal nerve and nucleus tractus solitarus. Third**,** ghrelin may directly affect hypothalamic nuclei by producing locally in hypothalamus (24). Ghrelin weakens the leptin-induced decline in body weight and food intake by regulating the expression of several hypothalamic peptides including NPY, AgRP and orexin (25) (26). It also has inhibitory effects on CORH producing neurons and POMC neurons (27). Ghrelin regulates the secretion of growth hormone by pituitary gland. Also ghrelin is shown to signal to initiate the meal for short term energy balance (28). This food intake after ghrelin administration is mediated by its stimulatory effects on emptying of stomach (29). Ghrelin may also play role in long term energy balance. Ghrelin administration in rodents increases the adiposity by decreasing the fat usage (30). Also ghrelin and BMI are negatively correlated, for example, ghrelin level increases when obese person loses weight or level decreases when anorexic person gains weight (31)(32).

Further, it is expected that in obese individuals that leptin level should decrease and ghrelin level should increase. However, the reverse is happens in reported cases, but it is still unclear whether it is because of the consequences or cause of obesity (33)(34). Obesity is associated with leptin resistance, leads to over-eating which causes increased levels of circulatory leptin. This pro-longed exposure to leptin damages the hypothalamus which in turn gets leptin resistant (35). Leptin resistance may also due to the defect in crossing blood-brain barrier (36). It is still not clear if the increased levels of leptin cause the decreased level of ghrelin, as there is no direct influence is found. The decreased ghrelin level can be due to the fact that body gets physiologically adapted to the positive energy balance related to obesity (34). Also there is any kind of signalling defect or resistance to ghrelin is not found (37).

The more common form of genetic variations is the single nucleotide polymorphisms (SNPs). In human genome, around 500,000 SNPs occurs into the coding region (38). Among these, those which can cause amino acid alterations are called non-synonymous SNPs (nsSNPs), which can be deleterious in naure. This can account for the variation in the functional variety of encoded proteins in the population (39). The main task of pharmacogenomics is to identifying this deleterious nsSNPs. Considering this, we performed in-silico analysis of the nsSNPs of leptin and ghrelin gene and recognize the potential deleterious mutations.

2. MATERIALS AND METHODS

2.1 SNP Data Mining: The data of the human ghrelin (GHRL) and leptin (LEP) gene were retrieved from web-based sources National Center for Biological Information (NCBI; http:/www.ncbi.nlm.nih.gov) and the Online Mendelian Inheritance in Man (OMIM; http://www.ncbi.nlm.nih.gov/omim). The other details of SNPs (like rs IDs, references) and protein sequences of both genes were retrieved from dbSNP-NCBI (http://www.ncbi.nlm.nih.gov/SNP/)and NCBI respectively. SNPs were selected by using filters which includes missense, nonsense, stop gained SNP.

2.2 Prediction of functional consequences of nsSNPs by SIFT: Sorting Intolerant From Tolerant (SIFT; http://sift.jcvi.org) is a software which predicts whether the amino acid substitutions can be tolerated or would have a deleterious impact on phenotypical and functional characterstics. The rsIDS obtained from NCBI for both genes were submitted as query in order to obtain homology searching. The threshold is ≥0.05 for the intolerance index (40).

2.3 Prediction of functional effect of non synonymous SNP by PROVEAN: Protein variation effect analyzer (PROVEAN; http://provean.jcvi.org) predicts whether the substitution of amino acid is deleterious or tolerated. It is based on the BLAST hits which have more than 75% global sequence identity clustered together and top 30 of such

clusters from a supporting sequence are averaged within and across clusters to generate the final PROVEAN score (41). Provean threshold is - 2.5; if below threshold, substitution will be deleterious else it will be neutral. User input involves Fasta sequence and amino acid substitutions.

2.4 Functional significance of substitution by PolyPhen-2: Polymorphism Phenotyping v2 (PolyPhen; http://genetics.bwh.harvard.edu/pph/) is an online tool which calculates the functional significance of allele substitution by Naïve Bayes classifier system. It qualitatively differentiates the mutations in three different classes, probably damaging (probabilistic score >0.85), possibly damaging (probabilistic score >0.15) or benign (remaining), corresponding to the pairs of false positive rate (FPR) and true positive rate (TPR) brinks, adjusted separately for HumDiv (10% & 18% FPR) and HumVar (19% & 40% FPR for probably and possibly damaging mutations, resp.). Input for PolyPhen was comprised of Fasta sequence and amino acid substitution information. (42)

2.5 Disease associated SNP prediction by PhD SNP: Predictor of human Deleterious SNPs (PhD SNP; http://snps.biofold.org/phd-snp/phd-snp.html is a SVM based classifier which predicts an amino acid substitution in disease or neutral class. User input for PhD-SNP includes protein sequence and amino acid substitutions (43)

2.6 Prediction of Phenotypic Effect of nsSNP by nsSNP Analyzer: nsSNP analyzer (http://snpanalyzer.uthsc.edu) is a web tool which predicts whether a non-synonymous SNP has a phenotypic effect or not. It also provides additional information about the SNP to facilitate the interpretation of results like structural environment and multiple sequence alignment. It uses information contained in MSA and 3D structure to make prediction. It predict the phenotypic effect (disease-associated vs. neutral) of a nsSNP by using a machine learning method called Random Forest, and extracting structural and evolutionary information from a query nsSNP (44).

2.7 Prediction of stability change by iMutant: A support vector machine based tool iMutant 2.0 (iMutant 2.0; http://folding.biofold.org/i-mutant/i-mutant2.0.html) predicts the change in the stability of the protein by a particular mutation. iMutant 2.0 can be utilized both as a classifier for prediction of the signs of the protein stability changes upon mutation and as a regression estimator for predicting the related change in Gibbs-free energy (ΔG) (45). User input includes protein sequence and amino acid substitutions.

2.8 Prediction of functional impact of mutation by mutation assessor: The server predicts the functional impact of amino-acid substitutions, such as mutations discovered in cancer or missense polymorphisms. Evolutionary conservation of the affected amino acid in protein homologs is the basis of the evaluation of its functional impact. The method has been confirmed on a large set (60k) of disease associated (OMIM) and polymorphic variants. The server maps each variant to both Uniprot and Refseq protein sequences (if possible). Uniprot IDs are used to extract information about domain boundaries (Pfam, Uniprot), annotated functional regions (Uniprot), protein-protein interactions (Piana). Refseq protein IDs are used to extract known alterations in cancer (COSMIC), SNPs (dbSNP) and known role in cancer (CancerGenes). The server determines domain boundaries (using Pfam or Uniprot) for the region with the variant and builds multiple sequence alignment using all Uniprot protein sequences or uses existing one from the repository. It is available at http://www.mutationassessor.org. (46)

2.9 Analyzing the molecular characterization of substitution by SNPeffect: The SNPeffect 4.0 (http://snpeffect.switchlab.org/) is a database used to predict the phenotypic impacts of human SNPs. For this, it provides various tools which predict different characterization of both coding and non-coding regions of a gene. **These** tools include TANGO (predict aggregation prone regions), WALTZ (predict amyloidogenic regions), LIMBO (predicts hsp70 chaperone binding sites), and FoldX (predicts the possible impact on protein stability). Further, SNPeffect focuses on molecular characterization, identification and explanation of disease variants in human proteome (47). User input involves the Fasta sequence and amino acid substitution.

2.10 Prediction of association of disease with substitution by MutPred: MutPred (http://mutpred.mutdb.org/) is a web tool developed to predict whether the amino acid substitution is disease-associated or neutral. It also predicts the molecular cause of that substitution. MutPred is based upon SIFT and a gain or loss of 14 different structural and functional properties. It was trained utilizing the deleterious mutations from the Human Gene Mutation Database and neutral polymorphisms from Swiss-Prot. It uses SIFT, PSI-BLAST (48), and Pfam profiles (49), also some structural disorder prediction algorithms, including TMHMM, MARCOIL (50), and DisProt (51). Functional analysis includes the prediction of DNA-binding site, calmodulin-binding targets, catalytic domains, and post-translational modification sites (52). User input involves Fasta sequence and amino acid substitutions.

3. RESULTS AND DISCUSSION

3.1 SNP Data Mining

The leptin and ghrelin gene that are under study were investigated for reported SNP's using dbSNP. For leptin, a total of 112 SNPs were reported in *homo sapiens* out of which 57 were found to be non-synonymous (55 missense, 2 stop gained) and For ghrelin, a total of 778 SNPs were reported in *homo sapiens* out of which 73 were found to be non-synonymous (70 missense, 3 nonsense).

3.2 SIFT

Sequence homology based SIFT program (ref.) was used for sorting the deleterious ns SNP from all the SNPs selected using dbSNP. The rsid's of all the nsSNP (57 for leptin and 73 for ghrelin) are submitted separately. Among 57 nsSNP of leptin only 6(rs1800564, rs17151919, rs75506045, rs76529182, rs104894023, rs111650508) were found out to be damaging. It was also found that out of these 6 , 4 rsIDSs(rs75506045, rs76529182, rs104894023, rs111650508) were found comparatively more damaging with a score of 0.00 and 1(rs17151919) was comparatively less damaging with a score of 0.01 and 1(rs1800564) was to be tolerated. Among 73 nsSNP of ghrelin only 3 (rs696217, rs4684677, rs34911341) were found to be damaging. It was also found that out of these 3, 2 rsIDs (rs4684677, rs34911341) were found comparatively more damaging with a score of 0.00 and 1 (rs696217) was comparatively less damaging with a score of 0.04.

3.3 Provean

The results obtained by SIFT for ghrelin when analyzed by Provean program, it was predicted that 2 rs4684677, rs34911341 of these nsSNP selected by sift were deleterious and 1 (rs696217) were predicted to be neutral. For leptin, 2 ns SNPs (rs76529182, rs104894023) were found deleterious and rests of the 4 (rs1800564, rs17151919, rs75506045, rs111650508) were predicted neutral. Thus, complimenting the results obtained by SIFT.

3.4 Polyphen 2

Polyphen v.2 provides a recheck on the SNP's predicted to be deleterious or damaging by using naïve bayes classifier. For leptin, Polyphen 2 predict 4 (rs75506045, rs76529182, rs104894023, rs111650508) to be damaging (2 were probably damaging (rs75506045, rs104894023), & 2 were possibly damaging (rs76529182, rs111650508)) and for ghrelin, Polyphen 2 predicts all 3 (rs696217, rs4684677, rs34911341) to be probably damaging with a score of 0.999,0.99 and 0.996, respectively.. Thus supporting the results predicted by SIFT and Provean.

3.5 iMutant

iMutant comments on the effect on the stability of a protein for a particular amino acid substitution resulting from the nsSNP. It calculates the free energy change (DDG). For leptin, iMutant program predicts all the 6 ns SNPs (rs1800564, rs17151919, rs75506045, rs76529182, rs104894023, rs111650508) led to destabilizes the structure of protein and for ghrelin it predicts 2 SNPs (rs696217, rs34911341) decreases the stability while 1(rs4684677) increases the stability of protein.

3.6 PhD-SNP

PhD-SNP is a SVM based classifier that classifies the nsSNPs as neutral or diseased. For leptin, PhD-SNP predicts 2 (rs76529182, rs104894023) nsSNP' to be disease and 4 (rs1800564, rs17151919, rs75506045, rs111650508) to be neutral. For Ghrelin, PhD-SNP predicts all 3 nsSNPs to be neutral.

3.7 nsSNP Analyzer

nsSNP analyzer analyzes nsSNPs on 3 aspects and predicts their structural and functional effect on the protein. For leptin, it predicts 4 (rs75506045, rs76529182, rs104894023, rs111650508) out of 6 to be disease.

3.8 Mutation assessor

Mutation assessor assesses the functional impact of a particular nsSNP using MSA between protein homologs and provides us a functional impact score (reva et al.). For leptin, mutation assessor predicts 5 (rs17151919, rs75506045, rs76529182, rs104894023, rs111650508) out of 6 to have a "medium" impact on the function of leptin with rs104894023 having the maximum impact score of 2.7. For ghrelin, mutation assessor predicts all 3 (rs696217, rs4684677, rs34911341) have a "medium" impact on the function of ghrelin with rs34911341 having the maximum impact score of 2.89.

3.9 SNP effect

SNP effect predicts the impact of nsSNP on aggregation tendency (TANGO), Amyloid –forming propensity (WALTZ), chaperone binding tendencies (limbo) and structural stability (foldX) of the protein. Analysis by TANGO, predicted that V94M (rs17151919) tends to decrease (dTANGO score of -85.62) while R105W (rs104894023) tends to increase (dTANGO score of 58.09) the aggregation tendency of leptin. For ghrelin, TANGO predicts that no mutation affects the aggregation tendency. All the nsSNP's under study shows no effects the amyloid propensity (as analyzed by WALTZ) and chaperone binding properties (as analyzed by LIMBO) of leptin. However in case of ghrelin, WALTZ predicts that mutation at Q89L (rs4684677) tends to increase amyloid propensity. LIMBO predicts none of the SNPs affects chaperone binding of ghrelin. Analysis by FoldX, predicts that V94M (rs17151919) tends to slightly enhance while R105W (rs104894023) tends to reduce the structural stability of leptin. No comments were obtained for ghrelin by FoldX.

3.10 Mutpred

Mutpred analysis comments on the likelihood of a mutation of being deleterious and also provides the top 5 functional effects that the mutation can have on protein. For leptin, mutpred predicts a high likelihood of missense mutation R105W (rs104894023) with P (deleterious) of 0.955 and K36R (rs111650508) with P (deleterious) of 0.657. For ghrelin, mutpred predicts a high likelihood of Q89L (rs4684677) with P (deleterious) of 0.628 and R50Q (rs34911341) with P (deleterious) of 0.581. it also provides an actionable

hypothesis and top 5 features that are the resultant of particular nsSNP. (Table)

4. CONCLUSION

Computational studies has proved to be a great allies to screen diseases specific SNP at molecular level. In this study in silico analysis has been performed to investigate the effect of nsSNPs on function–structure of leptin and ghrelin gene.

In leptin, out of 57 nsSNPs two point mutations in the coding region may have significant effect on leptin structure as well as in function. Similarly in ghrelin, 3 nsSNPs were found to have damaging effect on structure as well function. The computational analysis of free energy change due to mutation indicates that stability of leptin and ghrelin get decreased. Our result provides a significant computational approach to detect the pathologically significant nsSNPs in Leptin as well Ghrelin. Furthermore, the predicted disease associated nsSNP can be studied for the further development in potent drug discovery and to understand leptin ghrelin interface.

Tables

Table 1: effect of nsSNP on ghrelin

rsIDs	Amio Acid Change	SIFT	PROVEAN	PolyPhen	PhD-SNP	iMutant	Mutation Assessor
rs696217	L71M	Damaging	Neutral	Probably Damaging	Neutral	Neutral	Medium
rs4684677	Q89L	Damaging	Deleterious	Probably Damaging	Neutral	Neutral	Medium
rs349113	R50Q	Damaging	Deleterious	Probably Damaging	Neutral	Neutral	Medium

Figure showing the predictions by which the nsSNPs affect the ghrelin predicted by the respective softwares

Table 3: effect of nsSNP on structure and function of ghrelin by Mutpred and SNPeffect

rsID	substitution	SNPeffect			Mutpred
		tango	waltz	limbo	
rs696217	L71M	DNA*	DNA	DNA	Gain of disorder (P =0.1206) Gain of loop (P = 0.2754) Loss of stability (P = 0.3021) Gain of solvent accessibility (P = 0.5334) Loss of sheet (P = 0.1158)
rs4684677	Q89L	DNA	increase	DNA	Gain of stability (P =0.0432) Loss of disorder (P =0.0676) Loss of catalytic residue at Q91 (P = 0.0817) Gain of ubiquitination at K84 (P = 0.0856) Loss of sheet (P = 0.1398)
rs349113	R50Q	DNA	DNA	DNA	Loss of MoRF binding (P = 0.0115) Gain of methylation at K46 (P = 0.0921) Gain of helix (P = 0.132) Loss of glycosylation at P49 (P = 0.1688) Gain of relative solvent accessibility (P = 0.1894)

Figure showing predictons by which nsSNPs affect the structure and function of ghrelin. *DNA= Does Not AffectTable no 3: effect of nsSNP of leptin

rsIDs	Amino Acid Change	SIFT	PROVEAN	PolyPhen	PhD-SNP	nsSNP Analyzer	iMutant	Mutation Assessor
rs1800564	V110M	Damaging	Neutral	Benign	Neutral	Neutral	Decrease	Neutral
rs17151919	V94M	Tolerated	Neutral	Possibly Damaging	Neutral	Neutral	Decrease	Medium

rs75506045	Q160H	Damaging	Neutral	Possibly Damaging	Neutral	Disease	Decrease	Medium
rs76529182	W13L	Damaging	Deleterious	Possibly Damaging	Damaging	Disease	Decrease	Medium
rs104894023	R105H	Damaging	Deleterious	Possibly Damaging	Damaging	Disease	Decrease	Medium
rs111650508	K36R	Damaging	Neutral	Possibly Damaging	Neutral	Disease	Decrease	Medium

Figure showing predictions by which nsSNPs affect the leptin predicted by respective softwares

Table 4: Effects of nsSNPs on structure and function by SNP effect and MutPred

rsID	Substitution	SNPeffect				Mutpred	
		tango	waltz	limbo	foldX		
rs1800564	V110M	DNA*	DNA	DNA	DNA	Loss of ubiquitination at K115 (P =0.1216) Gain of helix (P = 0.2059) Gain of catalytic residue at V110 (P =0.4415) Loss of loop (P = 0.4786) Gain of disorder (P = 0.4856)	
rs17151919	V94M	decrease	DNA	DNA	enhance	Gain of disorder (P = 0.0673) Gain of glycosylation at P90 (P = 0.1361) Loss of sheet (P = 0.1398) Loss of stability (P = 0.3395) Loss of loop (P = 0.4412)	
rs75506045	Q160H	DNA	DNA	DNA	DNA	Gain of catalytic residue at M157 (P = 0.1342) Gain of disorder (P = 0.2159) Loss of helix (P = 0.3949) Loss of solvent accessibility (P = 0.4011) Loss of MoRF binding (P = 0.5331)	Loss of MoRF binding (P = 0.0115) Gain of methylation at K46 (P = 0.0921) Gain of helix (P = 0.132) Loss of glycosylation at P49 (P = 0.1688) Gain of relative solvent accessibility (P =
rs76529182	W13L	DNA	DNA	DNA	Na**	Gain of helix (P = 0.0696) Loss of sheet (P = 0.1907) Gain of relative solvent accessibility (P = 0.2363) Gain of solvent accessibility (P = 0.3418) Gain of loop (P = 0.4661)	
rs104894023	R105H	increase	DNA	DNA	reduce	Loss of helix (P = 0.1299) Loss of disorder (P = 0.1919) Gain of loop (P = 0.2045) Loss of stability (P = 0.236) Gain of catalytic residue at D100 (P = 0.5065)	
rs111650508	K36R	DNA	DNA	DNA	DNA	Loss of methylation at K36 (P = 0.0187) Loss of ubiquitination at K36 (P = 0.027) Gain of catalytic residue at K36 (P = 0.0932) Loss of sheet (P = 0.1158) Gain of MoRF binding (P = 0.1326)	

Figure showing nsSNPs affect the structure and function of leptin
*DNA= Do Not Effect, **Na= Not applicable

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