Computational analysis of a sequence variant p.N103K in leptin gene involved in obesity

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Abstract

Obesity and its associated health complications is a huge health burden worldwide. Genetic factors play a crucial role in determining an individual's predisposition to the weight gain and being obese.Leptin, a key appetite-regulating hormone derived from the white adipose tissue,

primarily acts on hypothalamic neurons to activate catabolic pathway and inhibit anabolic pathway, which can result in anorexia and weight reduction.Congenital leptin deficiency is a autosomal recessive disease characterised by hyperphagia and early-onset obesity.Genome wide association studies (GWAS) approach and their findings signified a number of genetic variants predisposing to obesity, which further needs the insilico analysis to confirm the damaging effect on protein structure and function. A missense variant (c.309C>A; p.N103K) in *LEPTIN* gene in Egyptian child, identified through Whole exome sequencing was selected form the literature for *In Silico* analysis. The pathogenicity of the variant was evaluated through computational tools including Mupro, Predict, Fathmm, provean, SNAP2. additionally, the secondary structure of the normal protein and solvent accessibility were checked through RaptorX tools. The 3-Dimensional Structure was constructed via biovia discovery studio. The energy minimization of mutant structure was carried out by SWISS PDB Viewer. In the present study, Molecular Docking were performed which predicted that there is drastic decrease in the stability of the mutant protein. The energy calculation also revealed that there is a difference of kj/mol which also indicates that the variant has significantly decreased the stability of protein consequently resulting in obesity.

Key words: *Insilico* Analysis, Obesity, Missense variants, Molecular Docking, *leptin*

Introduction

Obesity is a severe medical problem worldwide, which needs new approaches and recognized international consensus in treating diseases leading to morbidity. Obesity can be defined as when the quantity of body fat store increase and effect health negatively. One of the most dominant disorders affected human throughout the world is obesity(Shahid, et al., 2016). Twenty-first century one of the most health challenges considers childhood obesity. Obesity is the major people health issue due to which the rate of death and diseases are increases (Guh, et al., 2009).Obesity caused by genetic factors can be classified as monogenic (caused by a single genetic mutation), syndromic (associated with other phenotypes such as neurological development abnormalities or organ/system malformations), or polygenic (caused by the mutation of a large number of genes) (Hinney, Vogel, & Hebebrand, 2010; Rao, Lal, & Giridharan, 2014; Thaker, 2017). If obesity continues with the same rate till 2030 the range could rise to 2.16 billion obese and 1.2 billion overweight people, or 38% percent

and 20% of the world's adult population, respectively (Kelly, Yang, Chen, Reynolds, & He, 2008). The majority of adults in some regions are overweight. In the United States, 61 percent of all adults are overweight; in Russia, 54 percent are overweight; in the United Kingdom, 51 percent are overweight; and in Germany, 50 percent are overweight. In Europe as a whole, more than half of those aged 35 to 65 are overweight (Memedi, Tasic, Nikolic, Jancevska, & Gucev, 2013; Y. Wang & Lobstein, 2006).Monogenic obesity is caused by a mutation or deficiency in a single gene (Huvenne, Dubern, Clément, & Poitou, 2016).Mutations in genes that play a physiological role in the hypothalamic leptin-melanocortin energy balance system, such as leptin, leptin receptor, POMC, prohormone 1/3 convertases (PC1/3), and MC4R, are linked to the currently known monogenic forms of obesity.Obesity may cause other diseases including arterial hypertension, cardio vascular disease, diabetes mellitus, Dyslipidemia and cancer (Nguyen, Magno, Lane, Hinojosa, & Lane, 2008).The OB (obese) gene and its protein product, leptin(also known as OB protein), a 16000 MR cytokine-like hormone (Matson, 2000).It is typically in autosomal recessive disease characterised by hyperphagia and early-onset obesity. A sequence variant (c.309C>A; p.N103K) in the *leptin* gene cause obesity located on the chromosome 7q31.3,codes for 167 amino acid protein, has three exons and two introns(Isse, et al., 1995). Mutation that the aspargine are converted in to lysine. The *leptin* gene contain 4120bp which encodes 167 residues amino acid.The *leptin* gene showed that it encodes a hormone, that is expressed in adipose tissue and, at lower levels, in gastric epithelium and placenta(Friedman & Halaas, 1998).The *leptin* gene sequence variant (c.309C>A; p.N103K) to analyze its computationally by different bioinformatics tools.Leptin is a 16-kDa adipocytokine with 167 amino acids that is produced in relation to body fat levels. Agouti-related peptide (AgRP) and Neuropeptide Y(NPY) are both expressed by the first population of neurons , while the second expresses proopiomelanocortin (POMC) as well as cocaine and amphetamine-related transcripts (CART).There are two groups of arcuate (Arc) neurons in the hypothalamus. Leptin suppresses food intake and increases energy expenditure by acting on POMC/CART neurons to signal the depletion of energy reserves.Leptin inhibits AgRP/NPY neurons, and stimulating them (as in fasting) enhances food intake and energy conservation(Myers, Cowley, & Munzberg, 2008). The fact that circulating leptin levels rise during feeding and fall during fasting indicates that leptin serves as a physiological signal signalling the change from the state of energy sufficiency to hunger(Ahima, et al., 1996).

Materials and Methods

Sequence variant

To study the sequence variant which cause phenotypically the obesity. A syndrome in human being the sequence variant was packed from the previous research report study of the *Leptin* gene. The target variant of Leptin gene protein FASTA sequence was retrieved from the NCBI database with accession ID (P41159). The mutation was confirmed in the ensemble genome browser under the variant table of *Leptin* gene (Hunt, et al., 2018). For more studying, we confirmed the target variant through online tools that the affect of sequence variant on protein function and structure. Provean (Choi & Chan, 2015), Fathmm (Rogers, et al., 2018), Panther(Thomas, et al., 2003), Polyphen2 (Capriotti & Altman., 2011), FTSI (P. Kumar, Henikoff, & Ng, 2009), SNP&go (Montenegro, Lerario, Nishi, & Mendonca, 2019), Mupro to find the structural and functional consequence of variant (c.309 C>A resulting in to single amino acid p.N103K).

Phylogenetic Analysis

Phylogenetic as evolutionary tree to construct among *leptin* gene were compared with 14 other closed related species as identified by NCBI-BLAST program to check the maximum resemblance and confirmation of related gene family in other species. The MEGA X programme used Neighbour-Joining techniques to establish the phylogenetic relationship between protein sequences, ensuring the statistical significance of the tree(S. Kumar, Stecher, Li, Knyaz, & Tamura, 2018).

Structural analysis prediction and validation

The complete 3D structure of *Leptin* gene is not present in the online tools PDB (protein data bank). The *Leptin* gene retrieved the FASTA sequence from NCBI database. The multi protein template structure was predict through online web server Alpha Fold(Wei, 2019). The refining approach which has been tested CASP 10 method. For validation of 3D modeled structure of *Leptin* gene Ramachandran plot (Kleywegt & Jones, 1996) and ERRAT was generated with procheck to check the phi and psi angles. For more studying the, validated structure was refined through RAMPAGE (W. Wang, et al., 2016)and Galaxy refine (Shin, Lee, Heo, Lee, & Seok, 2014)tools. Pyton script was used for model refinement to pre-refine the modelled structure (Righetto, Biyani, Kowal, Chami, & Stahlberg, 2019).

Mutagenesis of *Leptin* and Comparison with Wild-Type *Leptin*

The 3-dimensional structure of the mutant protein from the wild type protein through biovia discovery studio and chimera (Pettersen, et al., 2004). The mutant structure was retrieved from the wild type protein. The interface take script based command from user and performs the function and graphical user interface is present. In the mutagenesis to show different rotamers in chimera. Each rotamers the mutation percentage valve which show how likely are the chances of each rotamers. The possible clashes in the structure was then cleared and remove. Both the wild type and mutant type protein structure were compared through biovia discovery studio.

Impact of Deleterious and Energy Minimization

To study the variant analysis, that will examine the mutation alteration lying the novel structure through Hope server (Venselaar, Te Beek, Kuipers, Hekkelman, & Vriend, 2010). Hope server was used to draw residue to residue comparison. The 3-dimensional structure energy minimization was carried out through SWISSPDB (Guex & Peitsch, 1997). Mutagenesis of leptin and comparison with wild type leptin.

Molecular Docking

For more studying, the *Leptin*gene were docking through different computational tools Pubchem(Kim, et al., 2016),autodockvina 1.5.7 (Trott & Olson, 2010),Discovery studio (Jejurikar & Rohane, 2021). Furthermore to check the reliability of molecular docking confirmation through Pymol (Yuan, Chan, & Hu, 2017). To study how two or more molecular structure are fit together. It is technique how a protein interact with small molecules (LIGAND).

Results

Sequence Variant Annotation

The *Leptin* gene sequence variant were retrieved from ensemble genome browser and Uniprot databases. The *leptin* gene identified a missense mutation (c.309C>A; p.N103K) located on the chromosome 7q31.3. The mutation of the results that the aspargine are converted in to lysine. For more studying various online tools were tools for the structural and functional annotation of sequence variant Provean is affected the protein sequence with a score as =-5.228 that the mutation can affect the disease. Panther affect the disease with a score as 0.5

that effect the protein structure. Polyphen2 indicate the probably damaging with a score as 1.000. SIFT is affect the protein function with a score as 0.00 mutation can the disease. SNP&GO was deleterious that affect the protein structure and cause the disease. Mupro affect the protein structure stability with a score as -1.28 (Table1).

Table 1: Effect of Sequence p.N103K on protein function by different tools with mutational score.

Mutation	SIFT	Polyhen-2	Provean	Panther	SNP&GO	PredictSNP
p.N103K	**Affect Protein Function**	**Probably Damaging**	**Deleterious**	**Possibly damaging**	**Disease effect**	**Disease effect**
Mutation Score	**0.00**	**1.00**	**-5.228**	**0.5**	**7**	**73% expected accuracy score**

Secondary Structure Prediction and Mutagenesis

The secondary structure prediction was retrieved from PDBsum tool. There are 5 helices,5 helix-helix interacs, 4 beta turns, 3 gamma turns, 1 disulphide. To further the secondary prediction to check the solvent accessibility through RaptorX tool of wild type and mutant type. The result were consistent which depicted that decrease and increase solvent accessibility of *leptin* gene causing obesity (Figure 1).

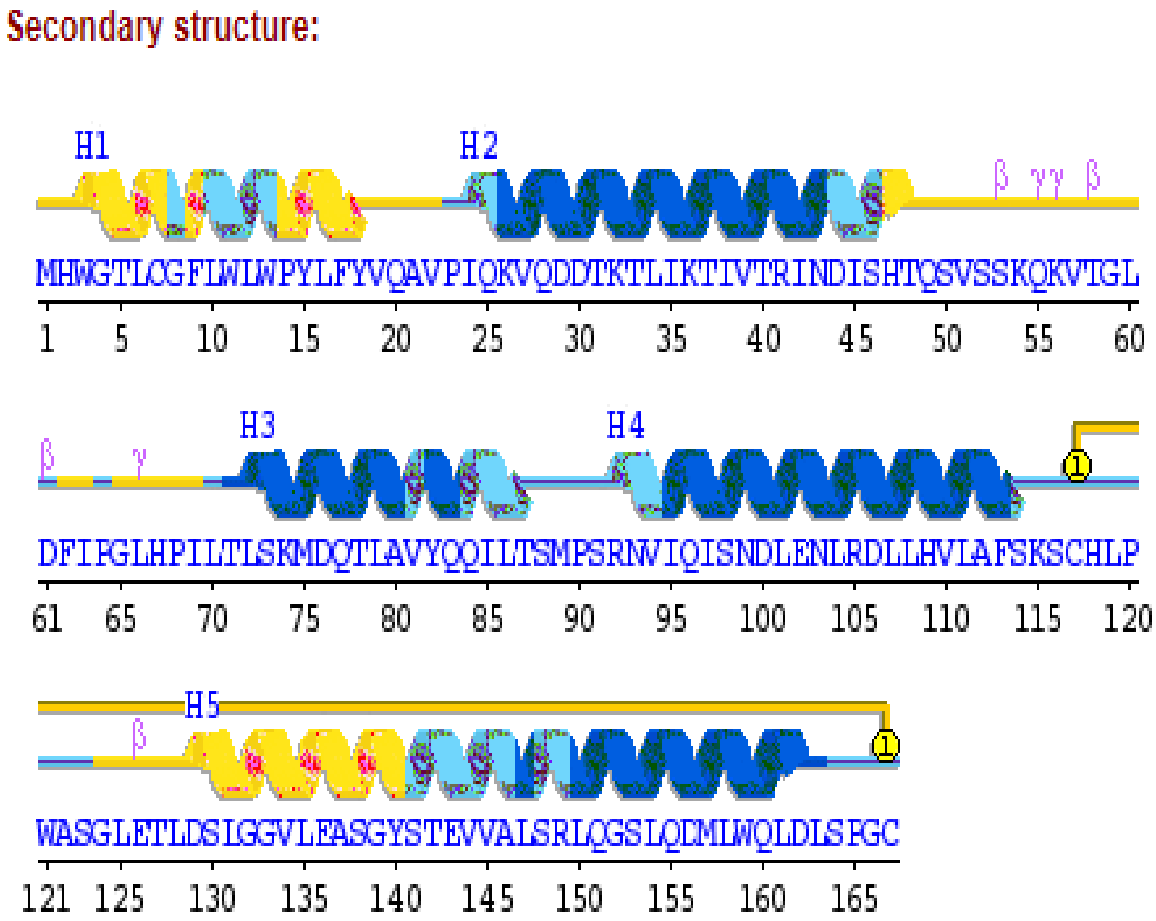

Figure 1: Complete normal secondary structure of leptin gene by PDBsum tool.

Phylogenetic Analysis

Based on BLAST results, the phylogenetic tree was also constructed to confirm the conservation leptin with these 14 different species listed in (Figure 2) belonging to the *leptin* gene family as highly conserved. The branch lenght show among the relation to which species are closely related to the human *leptin* gene. The *nomascus leucogenys, pongo abelli, gorilla, pan paniscus* species are closely related to the human leptin gene.

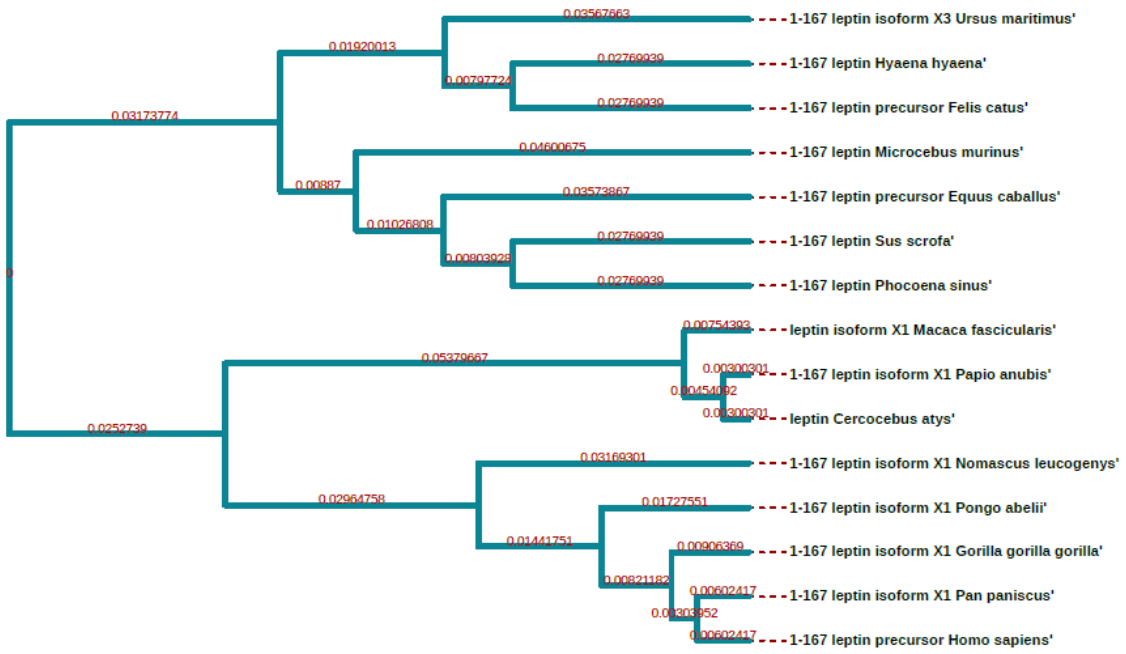

Figure 2: Phylogenetic tree analyses of 15 different protein sequences with different species using the Neighbor-Joining method by MEGA X with the branch length .

Structural Analysis Prediction and Validation

The *Leptin* gene protein sequence was retrieved from ensemble genome browser. The 3-Dimensional structure was obtained from online web based tool AlphaFold. Validation of 3-Dimensional structure through different tools that are use for to check the protein properties for structure validation. Ramachandran plot and EERAT were use for structure validation. Ramachandran plot show that 97.2% of residues are most favoured region and 2.7% residues in allowed region and no residues fall in the outlier region. ERRAT show that the quality factor score as 97.9%. To further enhance the reliability that the 3-D structure refine through online tool galaxy refine (Figure 3A).

Mutagenesis of *Leptin* and Comparison with Wild-Type *Leptin*

The mutant structure of *Leptin* aligned with the wild type of *Leptin*. Both the structure show that the difference between residues. Structure of mutant and wild type of the gene were minimized before comparison. Structural comparison depicted the difference in the side chain of the residues that Aspargine are conserved in to Lysine (Figure 3D). The comparison of residues that the wild type residues are smaller than the mutant type.To have a better view of

the mutation p.N103K and how it is changing the conformation of the mutant type from the wild type, the two structures were superimposed with the help of biovia discovery studio.The RMSD value of the structure has also been calculated by the Biovia discovery studio" and that is 0.00 and TM score (template modeling) as 1.00 which shows that the structure of the two proteins share the similar folds (Figure 3C).

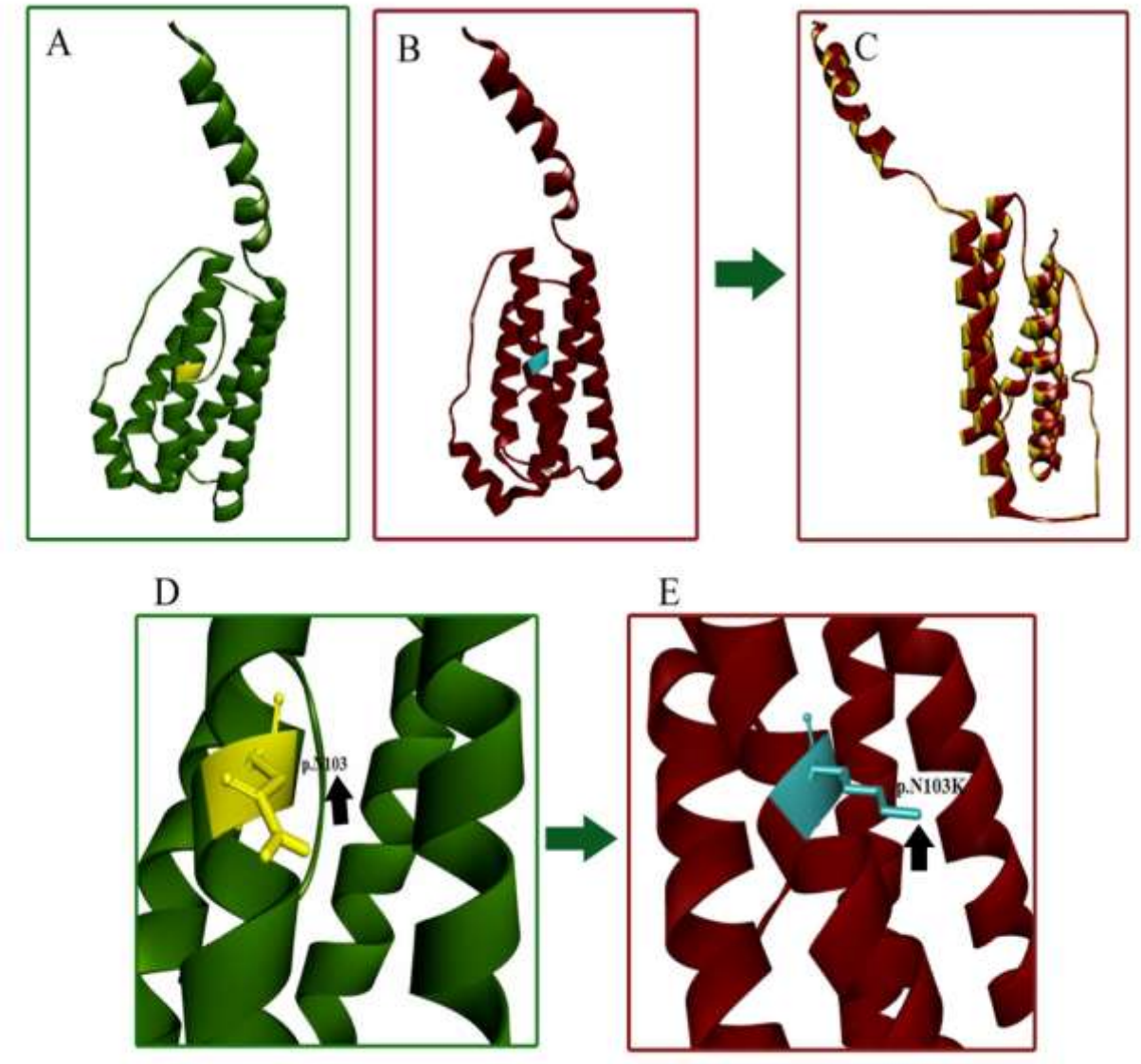

Figure 3: Structure analysis and modelled of normal and mutant leptin protein. (A) Structure of wild type leptin protein are highlighted in yellow colour. (B) Structure of mutant type leptin protein are highlighted in cyan colour. (C) Superimposition of wild (yellow) and mutant (red). (D) Wild type p.N103 residue are shown in yellow colour. (E) Mutant type p.N103K residue are shown in cyan colour.

Impact of Deleteroius and Energy Minimization

The *leptin*gene variant analysis check through the online Hope Server, demonstrated that the mutation occur in the single amino acid at the position of N103K. Each amino acid has its own specific size, charge and hydrophobicity value. The original wild type residue are newly introduced mutant residue ofter differ in these properties. Mutation region is bigger than the wild type region. The schematic structure of the original (Left) and mutant (Right) amino acid. The backbone of each amino acid is red and change the mutation backbone is black. These mutation is annotated with severity disease. The energy minimization of wild type and mutant type show that the protein is unstable that indicate the huge difference between wild and mutant type (Table 2).

Table 2: Total energy of wild type and mutant type structure after energy minimization.

Amino Acid Variant	Total energy after minimization kj/mol	Electrostatics constraint
Wild type	-5664.704	-3601.24
Mutant type	-5599.035	-3469.43

Molecular Docking

The 3D model structure of *leptin* predicted and characterized by online computational tools. Ramachandran plot and ERRAT show that approximately 97% residues area in allowed region. Molecular docking analysis the mutant type structure was obtained from chimera. For molecular docking analysis LIGAND molecules are retrieved from pubchem database with the ID of (LEPTIN H, LEPTIN E). The docking of wild and mutant type was conducted through Autodock vina server. The result show that the mutation of p.N103K change the structural confirmation of protein. Through molecular docking analysis binding of interaction with mutant type leptin gene. The following figures shows the docking result of both wild type and mutant type protein structure with the ligand molecules of LEPTIN H and LEPTIN E. (Figure 4A) shows the docking of the ligand molecule LEPTIN E with the wild type attached with residues GLN83, ARG105, ASP106 and TRP121, while the (Figure 4B) shows the ligand molecule LEPTIN E attached with residues GLU102, and TRP121 of mutant type

protein. (Figure 4C) illustrates that in wild type protein the ligand molecule LEPTIN H is attached with the residues LEU34, ASP100,and ASN103, while in case of mutant type (Figure 4D) the LEPTIN H is attached with residues LEU34, THR37, ASP100,and LYS103. The normal and mutant change in show ligand binding site atom and change its distance between the atom (Figure 5).

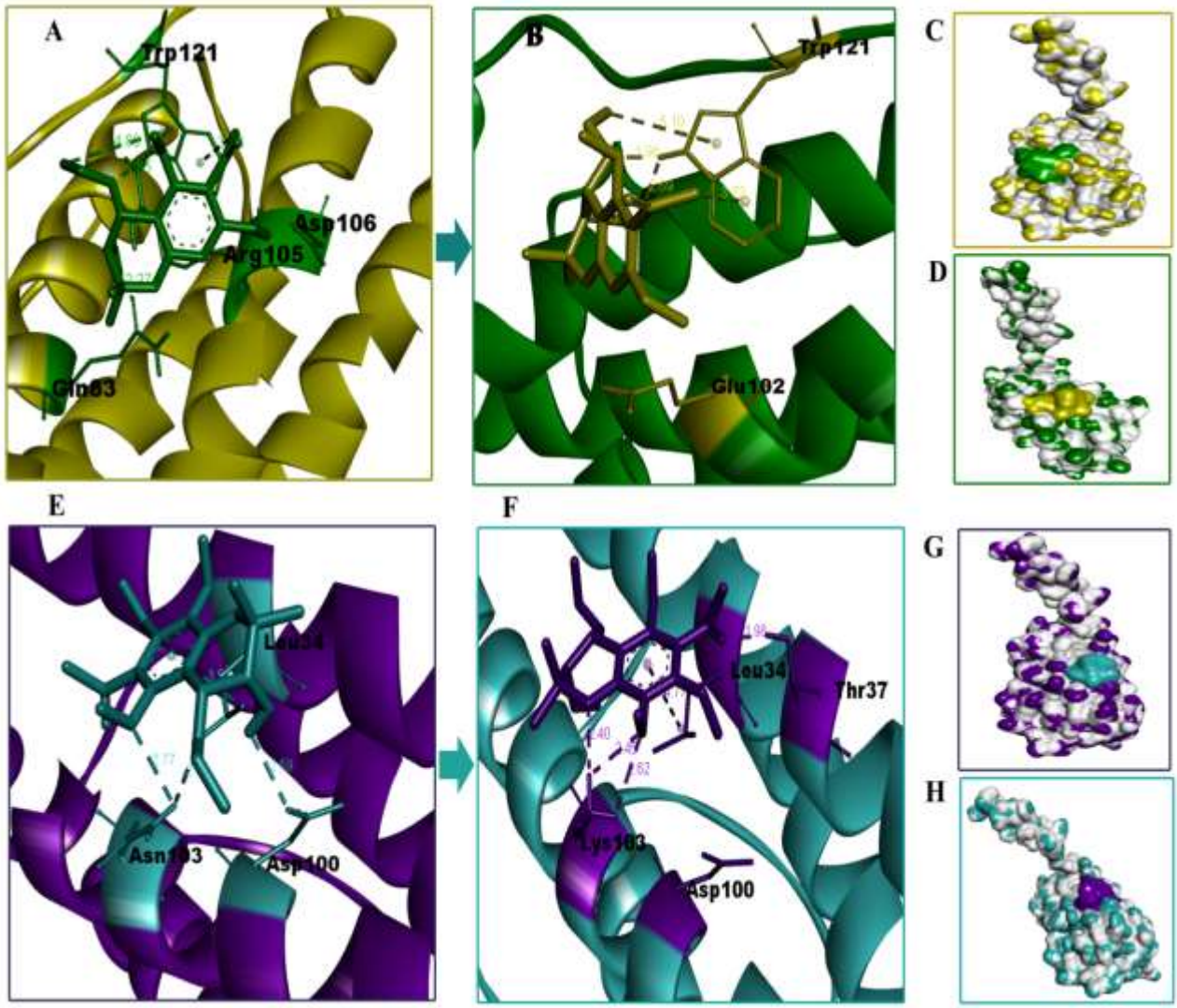

Figure 4: Visualization of the best docked leptin gene complex normal and Mutant. **(A)(B)** Cartoon view of the Normal and mutant leptin compound 1 (*LEPTIN E*) with H-bond interaction. **(C)(D)** Surface view are shown in atomic representation with normal and mutant. **(E)(F)** Cartoon view of the normal and mutant leptin compound 2 (*LEPTIN H*) with H-bond interaction. **(G)(H)** Surface view are shown in atomic representation with normal and mutant.

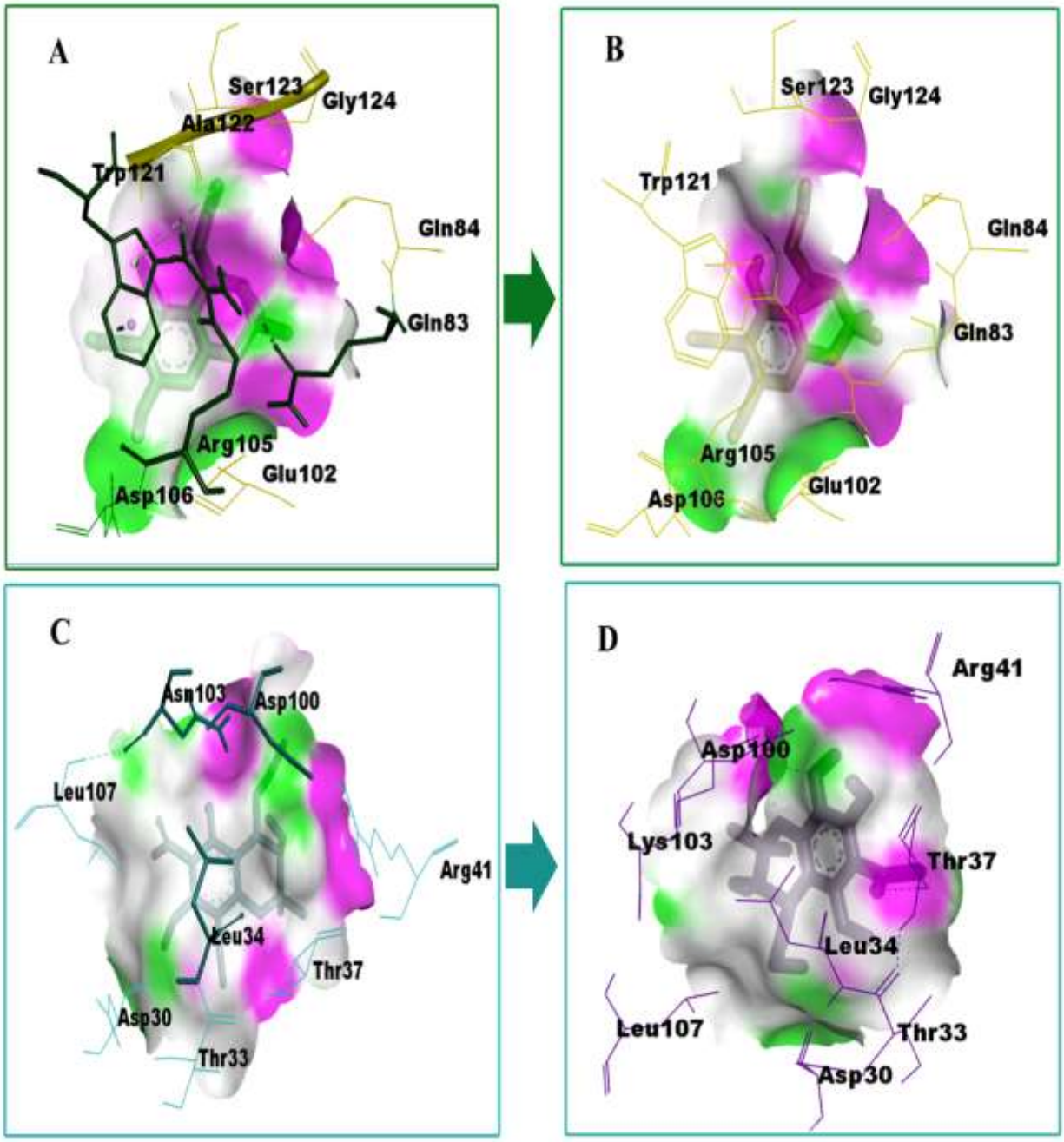

Figure 5: Visualization of the best docked binding site atom with normal and mutant. **(A)(B)** Normal and Mutant leptin with surface around ligand binding site atom with compound (*LEPTIN* E). **(C)(D)** Normal and Mutant leptin with surface around ligand binding site atom with compound (*LEPTIN* H).

Discussion

Obesity can be defined as when the quantity of body fat store increase and effect health negatively. One of the most dominant disorders affected human throughout the world is obesity(Shahid, et al., 2016).Obesity caused by genetic factors can be classified as monogenic (caused by a single genetic mutation), syndromic (associated with other phenotypes such as neurological development abnormalities or organ/system malformations), or polygenic (caused by the mutation of a large number of genes) (Thaker, 2017).Monogenic obesity is caused by a mutation or deficiency in a single gene (Huvenne, et al., 2016).The discovery of the leptin receptor (Lepr), encoded by the db gene followed soon after *leptin* was identified(Tartaglia, et al., 1995).Congential leptin deficiency is caused by mutation in the *leptin* gene that is located on chromosome (7q31.3) in the N-terminal region which can affect the function of protein in obesity. This gene has three exons separatedby two introns and codes for 167 amino acid protein(Isse, et al., 1995).To date ,only four pathogenic leptin mutations have been reported in obese children .The first mutation(p.Gly133fsX145) was detected in the *leptin* gene of two pakistani cousin with undetectable serum leptin levels.The second(R105W)was identified in four members of Turkish family (Wasim & Fakhar, 2015)(Strobel et al.,1998). The third(N.103K) in two Egyptian children (Mazen, El-Gammal, Abdel-Hamid, & Amr, 2009).The fourth(L72S) in a 14-year old female Austraian child(Fatima, et al., 2011).To study the sequence variant c.309C>A resulting in to single amino acid substitution p.N103K. The missence mutation occur in chromosome 7 at exon 3 varient ID rs 28954113. Which have 3448 base pair as well as 167 amino acid residue for protein. For further study of this varient c.309C>A in *leptin* gene.Leptin protein FASTA sequence is retrived from uniprot with the accession number (P41159).Apart from that available protein information and genome sequences now provides a better understanding of the corelation of the phenotype and genotype. This phenotype-genotype corelation studies has now been made easy by bioinformatics.In this study an effort is put into the identification of sequence varient which change the structure and function of the coded protein(Leptin protein). The sequence varient c.309C>A is evaluated through several tools to check the pathogenicity of the amino acid substituent. A change (c.309>A) resulting into P.Aspargine103lysine, is found to be disease causing sift, polyphine2, Panther, SNP&Go, Phd-SNP tools.The effect of the structure stability on single nucleotide change (C.103C>A) have been carried out in this study by several tools Like Provean and Mupro which predict that there is drastic decrease in the protein stability. PROVEAN also demonstrate that the

mutation at c.309C>A resulting into Single Amino Acid Sequence Variant p. Asp103 lys is deleterious with PROVEAN score as = -5.228. Amino acid substituent decrease the protein stability because mupro tool score is -1.2823877. Mupro score less than zero means the mutant decreases the protein stability; conversely, a score greater than zero means the mutant increases the protein stability. To show a structural analysis the confirmation of the protein were best docked.

Conclusion

In this study presented here, we have performed the Insilco analysis of a missense variant in *leptin* gene. The sequence variant (c.309C>A:p.N103K) is predicted to be disease causing by SNP&GO, Fathmm, Mupro, panther, provean. I-Mutant predicted that there is decrease in the stability of protein structure. The deleterious analysis and energy minimization exhibit that there is difference of kj/mol in the total energy of protein. The structure analysis of the mutant protein by pymol tool show that the aspargine ring is located at the center of strand while the substituted lysine is hanging outside. In molecular docking, the active binding site of mutant leptin is change the stability of the protein. In results we conclude that the variant (p.N103K) is changing the structural confirmation which results in decreased stability of protein causing obesity.

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AUTHOR CONTRIBUTIONS

S.I (Saqib Ishaq), A.Z (Abdul Aziz) and M.K (Muhammad Kashif) designed the study. S.I, M.K , M.I.H (Muhammad Inam Ul Haq) and O.H (Obaid Habib) performed various in silico analyses. R.T (Raheel Tahir). and S.I wrote the initial MS. M.A (Muhammad Afzal), Z.U.N (Zaib Un Nisa) and M.J revised the MS. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data that support the findings were derived from the following resources available in the public domain (GenBank) at https://www.ncbi.nlm.nih.gov/genbank/

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