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Missense Mutations in Leptin (LEP): A Bioinformatic in Computational Investigation of Three-Dimensional Protein Structure and Function

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Abstract

Introduction: A missense mutation arises when an amino acid change results from a nucleotide point mutation. Not all missense mutations result in significant protein modifications. An amino acid can be replaced by another amino acid with very similar chemical characteristics, allowing the protein product to function appropriately. Amino acid changes can occur in sections of the protein that have no effect on the protein's secondary-tertiary structure or function. A missense mutation can also create alterations in a protein's three-dimensional structure, rendering it non-functional.

Methods: Through a bioinformatic in computational investigation research of the three-dimensional protein structure and function of leptin protein completed in July 2023. This research analyze the missense mutation in the leptin (LEP) gene to the impact of leptin (LEP) mutations on protein structural change.

Results: We start with NCBI Web to locate the mutation in the Leptin gene, then move on to a variety of restriction enzymes for PCR-RFLP (Polymerase Chain Reaction Restriction Fragment Length Polymorphism) study, secondary structure modeling with Phyre2, and protein localization analysis with Uniprot. Using IP 2.0 to analyze signal peptides, TMHMM transmembrane proteins, and the Swiss model to analyze three-dimensional structures. **Conclusion:** Leptin missense mutations at the position in the 100th base where D changes to Y could be analyzed with the PCR-RFLP method using the AvaI enzyme on Leptin WT cut at 3 sites and Leptin MT cut at 4 sites. Leptin's missense mutation at the 100th base changes from D to Y, resulting in the three-dimensional protein structure change. Leptin was still expressed in the cell, but it cannot bind to the leptin receptor, and leptin cannot work.

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Introduction

Leptin is one of the adipokine members encoded by the LEP gene. The LEP gene encodes a non-glycosylated protein with a molecular weight of 16 kDa. Leptin is produced by various cells, such as adipocytes, skeletal muscle cells, the intestines, the brain, joint tissues, and bone tissues. Leptin is released into the blood circulation with central and peripheral effects and acts physiologically if it binds to the leptin receptor (LEPR) [1]. Leptin has a central role in appetite control and weight regulation homeostasis by inducing anorexigenic factors and suppressing orexigenic neuropeptides in the hypothalamus [2]. Leptin is known as the key sensor in the interconnected processes of energy metabolism and immune system regulation [3]. Leptin can reduce glucose uptake by enterocytes and subsequently exert an inhibitory effect on glucose absorption in the gut [4,5].

A missense mutation occurs in an amino acid change arising from a point mutation in a nucleotide. The type of non-synonymous substitution in the DNA sequence Not all missense mutations cause significant changes to the protein. An amino acid can be replaced by another amino acid with very similar chemical properties, so the protein product can normally function. Amino acid substitutions can occur in regions of the protein that do not significantly affect the secondary-tertiary structure and function of the protein [1]. A missense mutation can also cause changes in the three-dimensional structure of a protein that render it non-functional. This research will discuss the missense mutation in the leptin (LEP) gene through a bioinformatic in computational investigation of the three-dimensional protein structure and function of the leptin protein.

Bioinformatic computational investigation methods

This Bioinformatic computational investigation study was conducted in July 2023, using HP ENVY x360 13-ag0023AU amd ryzen 7 2700 8gb 1tb win 13", 8GB Memory RAM, 64-bit operating system, x64-based processor was to determine the effect of leptin (LEP) mutations on protein structure alteration. We found the mutation position in the Leptin gene on the NCBI web https://www.ncbi.nlm.nih.gov/. Selection of restriction enzymes for PCR-RFLP (Polymerase Chain Reaction Restriction Fragment Length Polymorphism) analysis using https://nc2.neb.com/NEBcutter2/. Secondary structure modeling with Phyre2 at http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index. Analyzing Protein location with Uniprot at https://www.uniprot.org/uniprot. Analyzing Signal peptide with IP 2.0 at http://www.cbs.dtu.dk/services/SignalP-2.0/#submission. Analyzing TMHMM Transmembrane Protein at http://www.cbs.dtu.dk/services/TMHMM/. Analyzing three-dimentional protein structure with Swiss model at https://swissmodel.expasy.org/. Visualization of three-dimentional protein structures and their interactions with Pymol analysis at http://pymol.org/academic.

Results & Discussion

Leptin was derived from the LEP gene located on chromosome 7th, with 167 amino acids [1,2]. Leptin function was highly conserved in all mammalian and non-mammalian leptins due to the presence of a tertiary three-dimensional protein structure that allowed for the formation of the disulfide bridges. Leptin was produced and secreted primarily from adipose tissue into the circulation [4]. Circulating leptin levels reflected the abundance of adipose tissue and the status of energy storage in the brain [3]. The expression of leptin and its amount in circulation showed fluctuations in circadian rhythms and also changed with the state of a person's nutritional status. Fasting conditions could reduce circulating leptin levels, while eating, satiety, and obesity could increase leptin levels [6,7].

From the NCBI data at http://www.ncbi.nlm.nih.gov/gene/3952, the LEP gene was located on chromosome 7q32.1. This gene encoded a protein that was secreted by white adipocyte cells into the blood circulation. Leptin circulated in the blood binded to the leptin receptors in the brain, which activate downstream signaling pathways that inhibit eating and increase energy expenditure [1]. Mutations in the LEP gene and its regulatory regions lead to severe obesity and obesity morbidity with hypogonadism in human patients [4,7].

A case study reported that the presence of a leptin mutation could lead to the absence of leptin in the circulation and cause extreme obesity, with early onset due to a novel homozygous transversion (c.298G → T) in the LEP gene, leading to a change from the amino acid aspartic acid to the amino acid tyrosine at position 100 (p.D100Y) (4). The data obtained from dbSNP indicated that the chromosome position in 128254557, with dbSNP code rs724159998, had a missense mutation in codon D (Asp) to Y (Asn) at position 100th, see **Figure 1.**

RFLP enzyme analyzed using Nebcutter on wild type (WT) and mutant leptin (MT) from http://nc2.neb.com/NEBcutter2/, see Figure S1.

From the restriction enzymes, we could analyze them based on the location of the mutation and the enzyme that cut in the sequence. The AvaI enzyme was determined to be a restriction enzyme, whereas in Leptin WT, it will cut at three locations into four fragments. In the Leptin MT, it would be cut at 2 locations into 3 fragments that could be detected by 2% agarose gel electrophoresis (see in Figure S2).

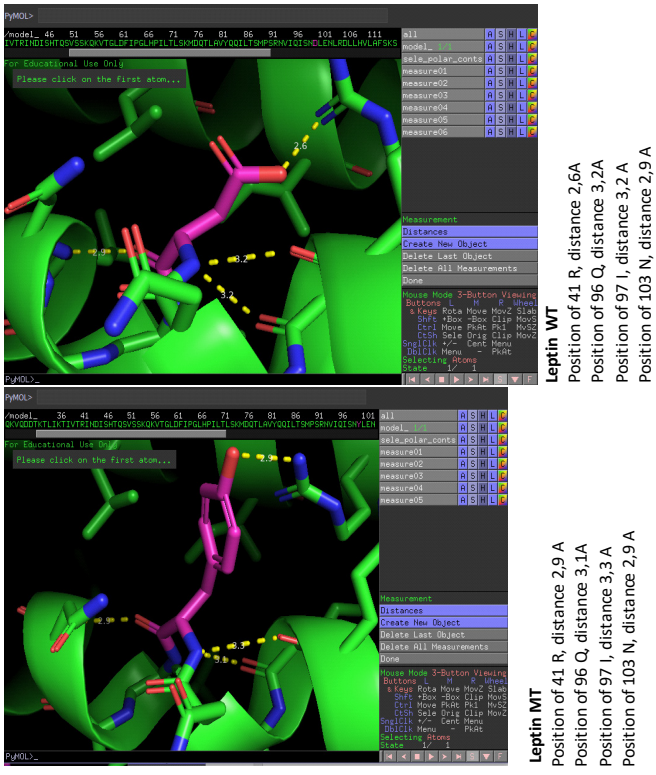

Figure 1: The three-dimentional structure analysis using Pymol. At the 100th base position, D changes to Y. There are changes in the position and distance between bases that cause changes in the three-dimensionality structure of the protein.

Based on the analysis using NEB cutter software, the AvaI restriction enzyme was chosen to be used for the PCR-RFLP method. Visualization results using 2% agarose gel electrophoresis for RFLP of the Leptin WT gene using the AvaI restriction enzyme would obtain four fragment cuts at 1198 bp, 1442 bp, 1884 bp, and 2059 bp. Meanwhile, RFLP of the Leptin MT gene using the AvaI enzyme would cut at 2 points, resulting in 3 fragment pieces at 170 bp, 1198 bp, and 2059 bp. Fragments below 100 pb were generally not identifiable. However, the difference in Leptin WT fragments that appear at 170 bp was enough to distinguish if there was a mutation of the Leptin MT gene. From this data, it was known that the PCR-RFLP performed can be used to detect leptin MT at the 170 bp position.

In humans, leptin consists of 167 amino acids with a long protein and a hydrophobic rounded fold with four helices. Leptin binded to a membrane protein receptor (LEPR), which consists of four cytokine receptor homologous domains (CRH). CRH had an Ig-like domain, a transmembrane segment, and a C-terminal cytoplasmic domain. Several shorter isoforms were found with multiple domains. Preliminary data on the identification of the leptin-binding domain of LEPR suggests amino acids at positions 323-640 in binding, consisting of an Immunoglobin-like domain and a second CRH (CRH2). The binding of the leptin protein to the LEPR would induce intracellular signal transduction via the JAK/STAT pathway through the phosphorylation of Tyr986 and Tyr1141 of the LEPR [8].

From the NCBI data, the nucleotide sequence data for Leptin was:

1-50 MHWGTLCGFLWLWPYLFYVQAVPIQKVQDDTKTLIKTIVTRINDISHTQS

51-100 VSSKQKVTGLDFIPGLHPILTLSKMDQTLAVYQQILTSMPSRNVIQISND

101-150 LENLRDLLHVLAFSKSCHLPWASGLETLDSLGGVLEASGYSTEVVALSRL

151-167 QGSLQDMLWQLDLSPGC

The location of the LEP gene mutation in rs724159998 at position 128254557, that there was a change of base D to Y at position 100, so that the Leptin nucleotide sequence becomes:

1-50 MHWGTLCGFLWLWPYLFYVQAVPIQKVQDDTKTLIKTIVTRINDISHTQS

51-100 VSSKQKVTGLDFIPGLHPILTLSKMDQTLAVYQQILTSMPSRNVIQISNY

101-150 LENLRDLLHVLAFSKSCHLPWASGLETLDSLGGVLEASGYSTEVVALSRL

151-167 QGSLQDMLWQLDLSPGC

The protein structure analysis was performed from UniProt: https://www.uniprot.org/uniprot/P41159. The signal peptide was found in proteins that were targeted to the endoplasmic reticulum and eventually destined to be secreted/extracellular/periplasmic/and others. Leptin was retained in the lumen of the endoplasmic reticulum, lysosomes, or other organelles along the secretory pathway or becomes a single-pass membrane protein [9]. The signal sequence was usually eliminated in the mature protein. Leptin was secreted into the bloodstream by adipocytes and its required for the maintenance of energy and body weight homeostasis. Leptin controled energy balance and body weight mainly by targeting LEPR-expressing neurons in the brain, especially in the hypothalamus. Signal Peptide Prediction at www.cbs.dtu.dk/services/SignalP-2.0/#submission. Signal Peptide IP.5.0 showed the final fate of the Leptin protein in the extracellular with a likelihood: 0.9654, showing that the leptin protein passes through the transmembrane and in the extracellular (Figure S3). Transmembrane α-helix prediction using

www.cbs.dtu.dk/services/TMHMM/TMHMM. This web showed that the leptin protein was not transmembrane, see in Figure S4.

The three-dimensional protein structure predicts protein using SwissModel Expasy with https://www.expasy.org/search/leptin. The three-dimensional structure of Leptin WT and Leptin MT was obtained in Figure S5.

Phyre2 analysis, about the percentage of alpha helix and beta strand of Leptin WT and Leptin MT Protein, see in Figure S6

The three-dimentional structure analysis of Leptin WT and Leptin MT Proteins with Pymol.

In the three-dimensional structure in Phymol analysis at http://pymol.org/academic turns out that in three-dimentional structure were change in the three-dimentional structure of the protein, **Figure 2**.

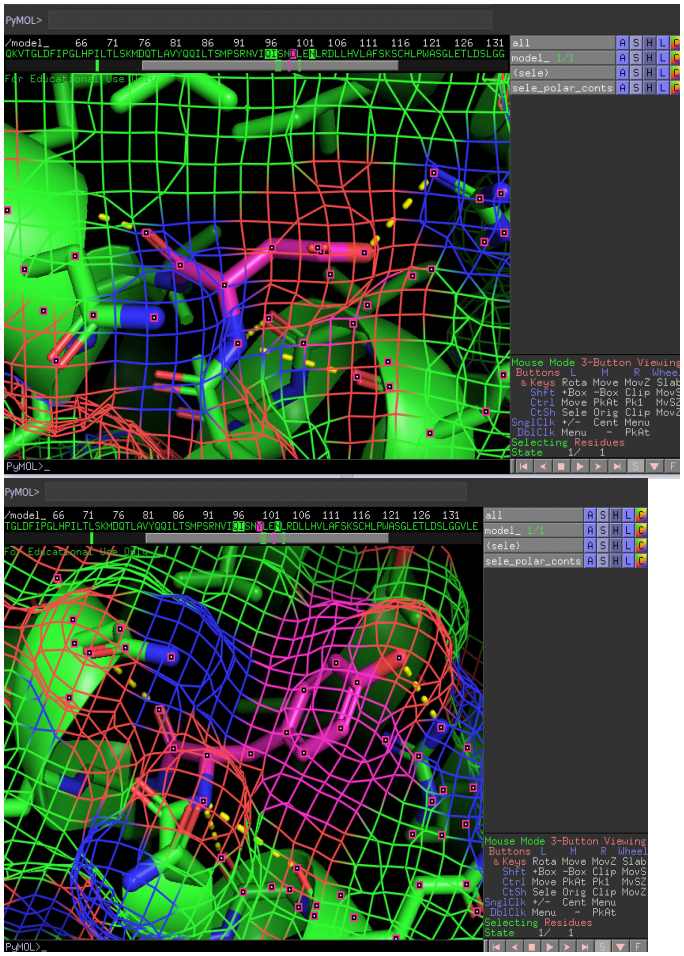

Figure 2: The three-dimensional protein structure view using Phymol, there are changes in the position and distance between bases that cause changes in the three-dimensionality structure of the protein.

A case of congenital leptin deficiency has been characterized by the absence of leptin expression or an absence of leptin in the circulating blood [4,7,10]. The mutant leptin protein was secreted but did not bind or activated the leptin receptor (LEPR) [4]. The mutant protein failed to reduce food intake and body

weight in leptin deficiency. A congenital leptin deficiency has been characterized by the presence of undetectable or very low leptin levels as a result of defects in either leptin synthesis or secretion [4]. Extreme obesity which early due to a novel mutation in the leptin gene, was accompanied by the production and secretion of a mutant protein that was biologically inactive. This was due to a missense mutation at position 100 from D to Y, which results in leptin deficiency and the early onset of extreme obesity [4].

Using bioinformatic computaional investigation methods regarding the three-dimentional structure and function of Leptin missense mutations, on the Pymol web, it could be seen that the three-dimentional protein structure of the leptin in leptin mutants has changed which causes a change in the leptin function, see in Figure S7, display of Leptin WT and Leptin MT proteins with their full structures. Leptin was still be expressed in the cell but when it`s in the blood circulation, leptin cannot bind to the leptin receptor, due to the three-dimentional protein structure change, finally, the function of leptin cannot work.

the limitation of our research was not using electrophoresis laboratory to determine leptin protein fragments.

Conclusion

Leptin missense mutations at the position in the 100th base where D changes to Y can be analyzed with the PCR-RFLP method using the AvaI enzyme on Leptin WT cut at 3 sites and Leptin MT cut at 4 sites. Leptin's missense mutation at the 100th base changes from D to Y, resulting in the three-dimensional protein structure change. Leptin was still expressed in the cell, but it cannot bind to the leptin receptor, and leptin cannot work.

Conflict of interest: None declared.

Data availability: All data generated and analysed in this article and Supplementary files.

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