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Integrative In-Silico and Evolutionary Analysis of the Human Leptin Receptor CRH2 Domain for Therapeutic Targeting

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Abstract: Leptin mediates various cellular processes through its receptor (OB-Rb) located on the cell membrane. The leptin receptor (LR) belongs to the Class 1 cytokine receptor family, characterised by four cysteine receptor homology domains (CRH). The three-dimensional structure of the leptin receptor has enhanced understanding of its atomic interactions with leptin. Further insights into the evolutionary significance and the relationship between leptin and its receptor improve therapeutic targeting of various diseases involving the leptin-leptin receptor axis. Therefore, in the present study, we aimed to analyse the evolutionary relationship of LR and to confirm its structure and function. The presence of four cysteine residues in the extracellular domain results in high peptide sequence homology among humans, monkeys, rats, and mice. The conserved leucine helps maintain the hydrophobic character of LR, and the disulfide bonds uphold the structural integrity of LR's binding sites. The extracellular domain of human LR comprises seven structural domains, including the conserved BOX1 and BOX II motifs. A detailed analysis of the molecular interaction between leptin and CRH2, along with the identification of key residues, aids in the selection of selective LR agonists and antagonists for therapeutic purposes.

Keywords: leptin; leptin receptor; CRH2 domain validation; mutation prediction

1. Introduction

Leptin, the adipocyte peptide hormone, is an essential component of energy homeostasis and body weight regulation, and its structure resembles that of the 4-α-helical bundle cytokines [1]. In mammals, adipocytes secrete leptin, and its blood concentration positively correlates with the amount of white adipose tissue [2]. Recently, it has become clear that leptin is a pleiotropic molecule with diverse and direct effects on various tissues [3]. It also influences many functions, such as angiogenesis, haemopoiesis, immune responses, and inflammation [4–7]. Leptin shows close structural similarity to IL-6, granulocyte colony-stimulating factor (G-CSF), and other long-chain cytokines, such as growth hormones [8].

Leptin binds to a membrane protein known as the leptin receptor (LR), encoded by the DB gene. It belongs to the class I cytokine receptor family. It consists of four cytokine receptor homology domains (CRH), an Ig-like domain, a transmembrane segment, and a C-terminal cytoplasmic domain in the long isoform [9]. The report indicates that the leptin receptor exhibits the highest sequence similarity to receptors in the IL-6 family and G-CSFR [8]. In humans, the extracellular part of the leptin receptor contains seven structural domains [10]. Domain 1 spans residues 62–178; domain 2 spans residues 235–328 and includes a fibronectin type III fold, forming a

cytokine receptor homology module (CRH) called CRH1. The third domain, covering residues 329–427, has an immunoglobulin (Ig)-like fold. Domains 4 and 5 include residues 428–535 and 536–635, respectively, both of which feature fibronectin type III folds and a second CRH, called CRH2. Domains 6 and 7 also adopt a fibronectin type III fold. The presence of an Ig-like domain between the two cytokine receptor modules resembles that of G-CSF and IL-6 family receptors [8]. Several studies have identified CRH2 as the receptor's primary high-affinity leptin-binding site [11]. The Ig-like domain is essential for JAK2 phosphorylation and the subsequent STAT3-dependent signalling [9,12].

Reports indicate that leptin binds to sites like those of G-CSF and IL-6 [3]. Binding site II was identified on the surface of helices A and C. In contrast, binding site III is situated near the N terminus of helix D. Site II interacts with the CRH2 subdomain of the OB-R. In contrast, residues in site III are likely to interact with the Ig-like domain of the leptin receptor [13]. In this study, we aimed to analyse the evolutionary relationship of LR and to confirm its structure and function. This approach also offers a pathway for designing therapeutic molecules that disrupt leptin binding and subsequent receptor activation.

2. Materials Methods

2.1. Sequence Analysis

Amino acid sequences of the leptin receptor (OB-R) from various species are obtained from the NCBI database along with their accession numbers. Then, conserved regions within these are identified using the CLUSTALW software. Finally, the amino acid frequencies for the leptin receptor are calculated using the ProtParam tool at https://web.expasy.org/protparam/ (accessed on 12 August 2010).

2.2. Secondary Structure Prediction

SOPMA software is utilised to predict the secondary structure of the leptin receptor across all listed species.

2.3. Hydropathy Plot

The hydropathy plot of the leptin receptor for all listed species was determined using the Kyte Doolittle hydropathy plot [14]. A window size of 19 was used to identify transmembrane regions in the protein. To identify amino acids that may be critical for OB-R's physical and chemical properties, we compared the differences between the hydropathy plots obtained with ProtScale, a computer programme on EXPASY (www.expasy.org (accessed on 12 August 2010)) that uses Kyte and Doolittle's software.

2.4. Functional Domain Identification and Phylogenetic Analysis

Using ScanSite (http://scansite.mit.edu/ (accessed on 28 August 2010)), we identified the functional domains of OB-R and examined their homology to assess functional similarity. The signal peptide, cytokine receptor homology (CRH) domains, immunoglobulin (IgG)-like domains, and BOX-1 and BOX-2 motifs were analysed for conservation using NPS (Network Protein Sequence Analysis) software. Additionally, a phylogenetic tree of leptin receptors from different species was computed using CLUSTAL-W.

3. Results

3.1. Multiple Sequence Homology of the Leptin Receptor

Amino acid sequences of the leptin receptor (OB-Rb) from various species were retrieved from the NCBI database, with accession numbers listed in Table 1. Conserved regions within the leptin receptor were identified using ClustalW2. Multiple sequence alignments aid in protein classification and analysis by revealing conserved sequences, active sites, secondary structures, and homologous regions. The basic features of Clustalw2, including simple gap insertion and deletion, offer further insights into the protein's functions and homology scores. The homology of OB-Rb amino acid sequences across different species was analysed, with conserved sequences highlighted in various colours (Table 1). These conserved features and identities were documented for each species. The differences suggest regions of functional importance and indicate conserved residues across all species, highlighting critical structural features. The length of OB-Rb sequences varies from the 57th amino acid in elephants to position 1165. Overall, sequence homology among species is high, with greater conservation observed in the extracellular region. Results show that amino acids 1 to 22 (signal peptide) are absent in goat, horse, camel, and elephant. Conversely, amino acids 1 to 14 are missing in bats, while amino acids 1 to 16 and 1 to 14 are absent in zebrafish and medaka.

Humans show the most significant homology with monkeys (95%) and elephants (92%) over a 57-amino-acid segment, followed by pigs (83%), cattle, dogs, and sheep (82%), and bats (81%). The homology percentages range from 17% to 79% across different species, as shown in Table 1. Sequence similarities are higher among closely related species. Interestingly, human peptide sequences are homologous to those of monkeys, rats, and mice, and other animals, such as sheep, goats, and pigs, also exhibit similarities. Additionally, zebrafish and medaka fish demonstrate homology comparable to that of turkey and chick, which share a high percentage of homology. Notably, four cysteines in the extracellular domain of OB-Rb are highly conserved across all species and form disulfide bridges.

Table 1. Accession number with frequency of amino acids (%).

		A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
1	Homo sapiens (human) AAB09673.1	2.7	3.3	4.5	5.7	4.8	3.9	2.6	6.8	6.7	**8.9**	2.1	5.7	5.9	3.6	2.2	**11**	6	7.4	2.3	3.9
2	Sus scrofa (pig) NP_001019758.1	3.3	2.8	4.4	6.2	4.6	3.9	2.9	6	6.4	**9.4**	2.1	5.1	5.9	3.6	2.7	**11**	6.4	7	2.4	3.8
3	Bos taurus (cattle) NP_001012285.2	3.5	3.1	4.9	5.8	4.5	3.6	2.6	6.4	6.6	**9.8**	1.6	5.3	5.8	3.9	2.6	**10.7**	6.2	7	2.3	3.7
4	Canis familiaris (dog) NP_001019805.1	2.7	2.9	4.2	6	4.5	4.1	2.7	6.3	6.7	**9.8**	1.8	5.3	6	3.5	2.7	**10.4**	6.8	7.4	2.4	3.8
5	Macaca mulatta (monkey) AAF34683.1	2.8	3.4	4.6	5.6	5.1	4	2.6	6.5	6.8	**9.1**	2.1	5.9	5.9	3.5	2.5	**10.5**	5.9	7.4	2.3	3.4
6	Mus musculus (mouse) AAC52705.1	4.2	3.3	4.7	5.7	4.3	4.4	2.2	5	5.5	**10**	2.1	5	6.4	4	3.2	**10.8**	5.5	8.2	2.3	3.3
7	Rattus norvegicus (rat) AAB06616.1	4.2	3.3	4.8	5.3	4.2	4.5	2.2	5	5.6	**10.2**	2.2	5.8	6.2	4.1	2.9	**10.2**	5.7	7.9	2.3	3.4
8	Myotis lucifugus (bat) AAU47264.1	3.7	3	4.6	5.7	4.4	4.2	2.4	6.8	6.7	**9.7**	1.8	5.7	5.4	3.7	2.6	**10.6**	5.8	6.7	2.3	4
9	Meleagris gallopavo (turkey) AAG40323.1	5.4	3.3	4	6.4	3.7	4	2.8	4.6	4.4	**10.1**	2.1	4.9	5.8	3.7	3.9	**11.4**	6.1	7.3	2.5	3.5
10	Gallus gallus (chick) NP_989654.1	5.3	3.4	3.7	6.4	3.7	4.2	3	4.6	4.4	**10.4**	2.1	5.5	5.7	3.8	3.6	**11.1**	6.1	7.2	2.4	3.3
11	Xenopus tropicalis (African clawed frog)	3.6	3.4	5	6.2	5.2	4.3	3.1	5.7	6.1	**8.8**	2.5	5.2	5	4.4	2.8	**9.6**	5.7	7.2	2.3	4
12	Danio rerio (zebra fish) NP_001106847.1	5.5	2.9	4.6	8.7	3.8	4.8	1.8	4.2	4.9	**8.8**	2.3	4.4	5.3	4.7	4	**11**	5.7	7.3	2	3.1
13	Oryzias melastigma (Indian madaka fish) ABC86922.1	5.1	2.7	4.3	7.5	3.2	4.3	2.9	4.1	5	**8.7**	2.7	4.9	6.9	4.7	3.8	**11.4**	5.1	6.8	2.3	3.5
14	Ovis aries (sheep) NP_001009763.1	3.4	3	4.7	4.5	4.2	3.7	3	6.8	7.2	**10.1**	1.7	5.5	5.8	3.5	2.6	**9.9**	5.5	8.2	2.8	4.1
15	Capra hircus (goat) AAW31714.1	6.1	3.5	5.3	9.6	3.5	5.3	0.9	5.3	7	**7**	0.9	2.6	3.5	4.4	2.6	**18.4**	8.8	1.8	1.8	0.9
16	Equus caballus (horse) AAD31284.1	5.9	3.9	3.9	9.8	3.9	5.9	2.9	7.8	5.9	**4.9**	0	2.9	4.9	5.9	2.9	**21.6**	3.9	1	1	1
17	Camelus dromedarius (camel) AAO91912.1	3.4	3.4	5.1	11.1	6	4.3	1.7	6.8	5.1	**8.5**	0	4.3	5.1	5.1	1.7	**20.5**	4.3	1.7	0.9	0.9
18	Elephas maximus (elephant) AAD00769.1	3.5	8.8	3.5	7	0	3.5	5.3	7	1.8	**7**	1.8	8.8	0	3.5	3.5	**7**	10.5	7	1.8	8.8

3.2. Frequency of Amino Acids

It is well known that the primary, secondary, and tertiary structures of leptin receptors are highly conserved across species. The conservation in amino acid sequences, their frequency of occurrence, and the secondary structure have been analysed from 18 species, including humans, monkeys, cattle, pigs, dogs, mice, rats, chicks, turkeys, bats, sheep, goats, horses, camels, Asiatic elephants, frogs, zebrafish, and medaka fish. Table 1 shows the amino acid frequency distribution in leptin receptors from these species. The amino acid serine (S) exhibits the highest frequency among them, except for the elephant. Other amino acids, such as leucine (L) and valine (V), have the second-highest frequencies, while glutamic acid (E) ranks third. Methionine (M) is the least common amino acid in all species except camels, horses, and chicks. The second least common is tryptophan (W), followed by arginine (R), histidine (H), and alanine (A). Leucine is the most abundant amino acid residue contributing to the hydrophobic character of the leptin receptor.

3.3. Peptide Sequences

Peptide sequences for the first 21 residues have been analysed across 18 species (Table 2). The analysis shows that in all species, N-terminal sequences start with methionine, except in bats, where phenylalanine (F) is the initial amino acid. The letter 'G' indicates the starting amino acid for the horse, 'Y' for the Asiatic elephant, and 'T' for the goat. Cysteine residues are highly conserved in the extracellular domains of humans, pigs, monkeys, rats, mice, and dogs, but in cattle, sheep, and goats, they are replaced by serine. The charged residue arginine (R) is absent in the first 21 amino acids of the N-terminal region, except in frog, bat, zebrafish, medaka fish, and the Asiatic elephant.

Interestingly, in monkeys, a conserved tyrosine is replaced by cysteine. All species, except the zebrafish, show a notable absence of arginine residues. Both positively and negatively charged residues are found in the C-terminal region of all species. Proline (P) is highly conserved across species and is considered structurally vital for folding properties. Interestingly, the N-terminal region of all species contains an odd number of Cys residues, which may facilitate disulfide-linked interactions with other receptor chains. There is also an odd number of Cys residues in the distal membrane domain of LR, suggesting the potential formation of an intramolecular disulfide bond to stabilise the ligand-binding site.

Table 2. Alignment of N-terminal amino acid sequences.

Species Name	Amino Acid Sequences
Sheep	MISQKFCVVLLH--WEFIYVTTA-21
Cattle	MISQKFCVALLH--WEFIYVITA-21
Human	MICQKFCVVLLH--WEFIYVITA-21
Monkey	MICQKFCVVLLH--WEFICVITA-21
Pig	MTCPKFSVALLH--WEFIYVITA-21
Dog	MTCQKFCVALLH--WEFIYLTTA-21
Mouse	MMCQKFYVVLLH--WEFLYVIAA-21
Rat	MMCQKFYVVLLH--WEFLYVITA-21
Bat	FIYVITAFNLAYPSTPWRFKL-----21
Chick	MYHQIILTMSLL-LGFLHVAAA-21
Turkey	MCHQIILTMSVL-LDFLHVAAA-21
Frog	MFWHWILPFFLL-LRYMQITAA-21
Horse	GKGSICISDQCSSAQFSEAES-21
Camel	GSICISDQCSSAQFSEAESTD-21
Zebra	MFFSDVLSCPPR--SVFIMLALL-21
Asiatic	YNAVYCCNEHECHHRYAELYV--21
Madaka	MWRINHSSTSVLFKRSQIGFR-21
Goat	TSWKNKDEMVPTTTDALLLTT-21

3.4. Disulfide Bonds

Cysteines are highly conserved in the leptin receptor and are essential for maintaining the structure needed for ligand binding. Humans and cattle share similar disulfide bridges (cys90–cys99, cys131–cys142, cys188–cys193, cys436–cys447, cys473–cys488, cys604–cys613). Mice and rats display similar disulfide bridges (cys3–cys37, cys89–cys118, cys90–cys99, cys131–cys142, cys188–cys193, cys434–cys445, cys602–cys611, cys851–cys879, cys973–cys977, cys953–cys958, cys496–cys526). Of the 15 disulfide bonds, 11 are conserved at Cys193 in both mice and rats. Three disulfide bridges, such as cys90–cys99, cys131–cys142, and cys188, are highly conserved among humans, mice, and rats, but not among monkeys and bats, which exhibit different disulfide bonds. These disulfide bonds are responsible for maintaining the structural integrity of receptor-binding sites rather than supporting their conformational stability.

3.5. Secondary Structure Analysis

The secondary structure of the selected 18 species was analysed using the SOPMA tool. A detailed examination indicates that the most prevalent structure is a random coil, except in elephants, which possess an extended strand interspersed with alpha-helices and a small percentage of beta-turns (Figure 1). Homologous regions among the leptin receptors suggest a similar secondary-structure pattern across these receptors. The structural alignment reveals standard features among these species, particularly the abundance of random coils, β-sheets, and α-helices. Analysis shows that the N-terminal domain contains seven β-sheets, resembling fibronectin–like structures found in cytokine receptor modules. These β-sheets are close to the ligand-binding site and the alpha chain, forming a disulfide bond between the alpha and β chains upon leptin binding. The presence of β-sheets followed by α-helices near the ligand-binding site is conserved across species. We propose that the overall structure of the N-terminal domain may be key to forming a stable high-affinity receptor complex. The predicted secondary structures were analysed for their motifs and residues, noting which are conserved across the 14 species and within different regions of the leptin receptor.

The amino acid residues (62–78) in the CRH1 domain are helical, while those from 235–328 adopt coiled and β sheet configurations. The CRH2 domain contains four conserved cysteines, a proline-rich hinge region, and a conserved spiral structure of the "WS X WS" sequence motif. The Ig-like domain includes β-sheets, turns, and helices. The WS X WS motif is highly conserved across all species and is critical for ligand recognition and signal initiation. It also contributes to receptor internalisation and protein stability. Structurally, this region consists of two fibronectin type III modules, each approximately 100 amino acids long, with seven β-strands folded antiparallel to form a barrel-shaped structure. A hinge region connecting the two barrel-like and type III modules contains the WS X WS motif and is predicted to function as a ligand-binding site.

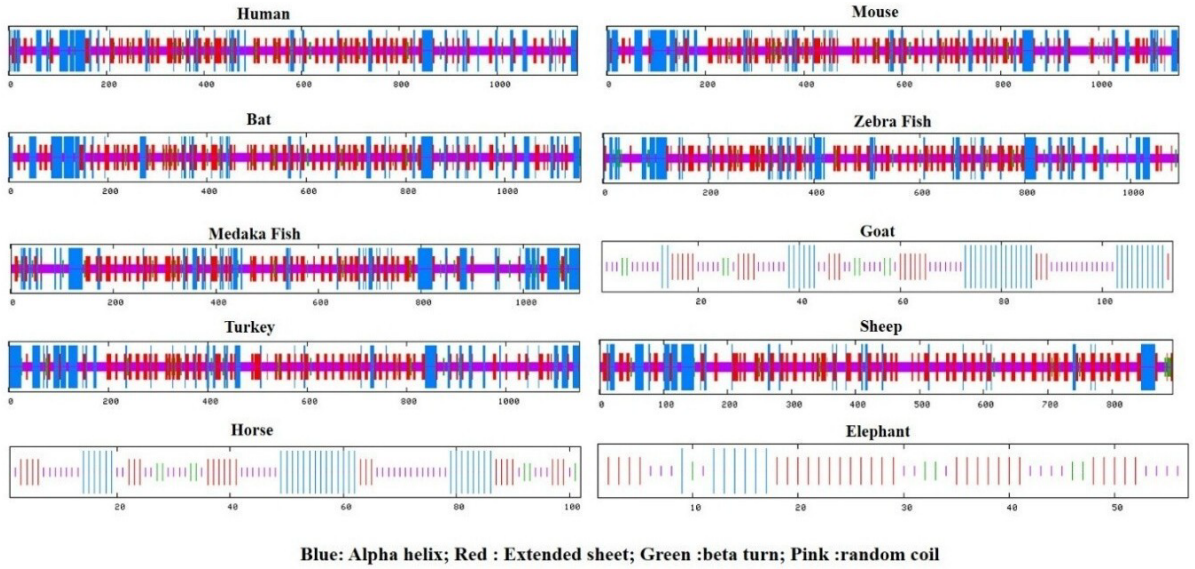

Figure 1. Secondary structures of the Leptin receptor from the chosen species. The structure is generated using the SOPAMA tool. Random-coil structures are dominant in all species except elephants.

3.6. Hydropathy Plot

A protein consists of several amino acids connected by peptide bonds. Each amino acid has a specific R group that determines whether the protein is hydrophobic or hydrophilic. The hydrophobicity of amino acids affects their placement in the final protein structure [14]. In our study, a hydropathy plot for OB–R from different species was created using a window size of 19. Peaks with scores above 1.6 were identified as transmembrane regions (Figure 2), observed in human, mouse, rat, bat, cattle, and monkey. Several peaks indicating hydrophilic areas (below the midline) and hydrophobic regions (above the midline) were detected. Interestingly, four species—the camel, goat, horse, and elephant—lacked transmembrane regions and had negative hydropathy values, indicating a hydrophilic nature. Additionally, the number of amino acids varies between 50 and 120 across different sequences, compared to 1165 in humans, mice, monkeys, pigs, and others (ranging from 1165 to 1092). Notably, the greater number of amino acid peaks in the hydrophilic region suggests the protein's capacity to interact with solvent molecules. The residues in the receptor beyond amino acid 800 are hydrophobic and originate from the cytoplasm.

In Figure 2, the hydropathy plots of the N-terminal region of the OB-R exhibit a consistent pattern in how transit peptides are organised. Besides the similarity of the plots, all transit peptide sequences appear to have similar slopes along their zig-zag lines. Additionally, some fragments from this region are notably alike. For example, each sequence in the N-terminal region is highly hydrophilic in zebrafish, whereas medaka fish display hydrophobic characteristics. There is a transmembrane region in LR between residues 800 and 900, which is conserved across all species. Interestingly, the amino acids in this region are highly conserved among species. Overall, the similarity between hydrophobicity plots matches amino acid sequences, and hydrophilic peaks occur at the same amino acid positions in zebrafish and medaka. Valine, with a hydropathy scale value of 4.2, is very hydrophobic, while Alanine is moderately hydrophobic with a value of 1.8. Conversely, serine has a mildly hydrophilic value of −0.8. Notably, no transmembrane region is identified in horses, camels, frogs, dogs, or elephants. The residues in these species may be hydrophilic. The number of amino acids within these regions is small and may be directly involved in ligand transport rather than signal transduction. In species where predicted transmembrane domains were absent, the corresponding sequences were often truncated or incomplete; therefore, no evolutionary inference regarding transmembrane domain loss was drawn from these entries.

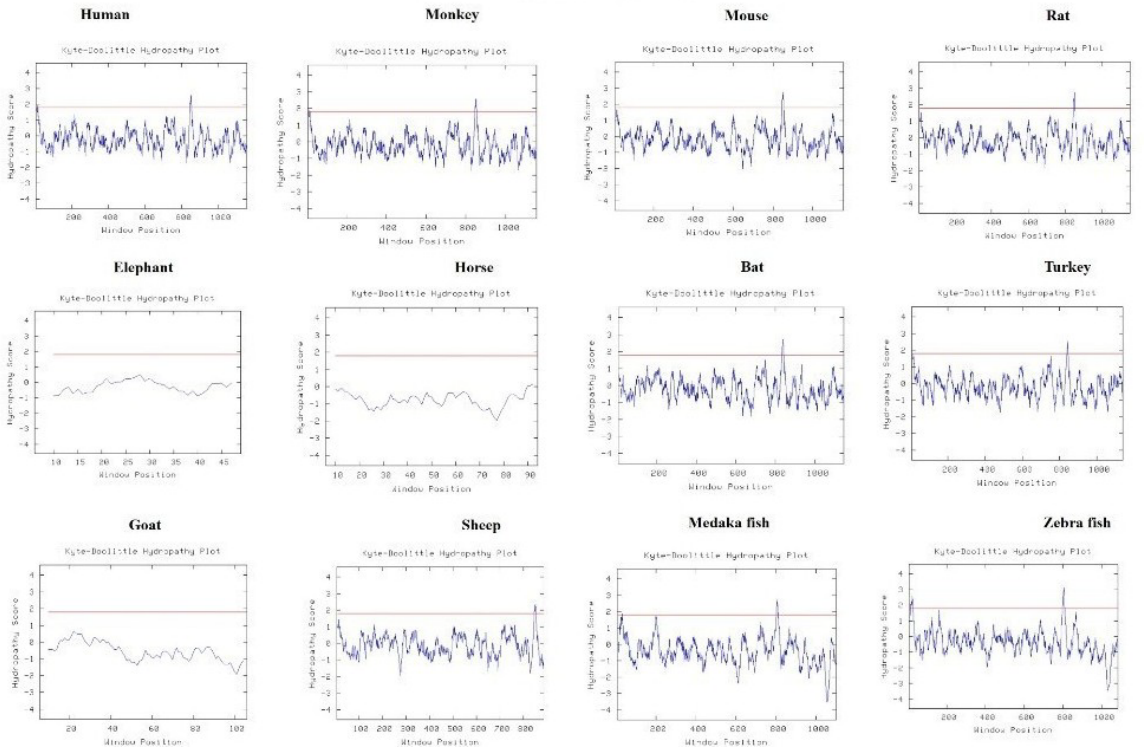

Figure 2. The hydropathy plots of selected species for identifying transmembrane regions. These regions are observed in humans, mice, rats, bats, cattle, and monkeys.

3.7. Extracellular Domains

The human LR encodes a protein of 1165 amino acids, including a 21-amino-acid signal peptide, an extracellular region of 800 amino acids, and a long cytoplasmic domain of 300 amino acids. The extracellular region of the OB–Rb in humans shows 75% homology with that of mice and 95% with that of the monkey. The extracellular part of the human LR contains at least seven structural domains. Domains 1 (residue 62–78) and 2 (residue 235–328) have a fibronectin type III fold and together form a cytokine receptor homology module (CRH), named CRH1. Domain 3 (residue 329–427) displays an immunoglobulin (Ig)-like fold. Domains 4 (residue 428–535) and 5 (residue 536–635) also exhibit a fibronectin type III fold and form a second CRH, called CRH2. Domains 6 and 7 adopt a fibronectin type III fold. The human leptin receptor sequence shows conserved extracellular, transmembrane, and intracellular domains. Homologies of the extracellular domain are higher in other species, such as pigs (85%) and mice (55%). Homologies of the transmembrane domain are higher in pigs, rats, and mice, sharing 90%, 86%, and 86% with humans, respectively. The human signal sequence resembles that of pigs but is less similar to those of rats and mice. The putative leptin-binding region, CRH2, is highly conserved across species. Notably, the sequences of leptin receptors, known as Box I and Box II motifs, are highly conserved and are suggested to play an essential role in leptin signalling.

3.8. Box I and Box II Motifs

The Jak2 tyrosine kinase is linked to the IL-6 receptor and is essential for activating cytokine signalling pathways. The membrane-proximal region of cytokine receptors that interact with Jak2 contains a highly conserved motif called Box I, which is a proline-rich sequence located after the transmembrane domain. The amino acid residues within the Box I motif are highly conserved across 14 species (see Table 3). The goat, horse, camel, and elephant lack this motif because they have fewer amino acids (57–117) than other species. Residues at positions 870, 872, 874, 877–880, 883–886, and 899 are identical in all species. Residues at positions 871, 881–882, and 889 are highly conserved, whereas those at 873, 875, and 876 are moderately conserved. Among the 14 species, turkey and chicken display identical sequences. Nonetheless, all 14 species show 100% homology. The sequence homology indicates that residues such as K, L, W, P, V, N, C, S, A, Q, and G are highly conserved across all species, although K is replaced by N in medaka fish and zebrafish.

The amino acid residues of the Box II motif are also analysed across different species, but they are not as well conserved as those of the Box I motif (Table 3). The similarity in amino acid residues among the organisms follows the same pattern as observed in the Box I motif. The chick and the turkey show more notable similarities,

while humans, pigs, sheep, dogs, cattle, and monkeys are moderately similar. The medaka and Zebrafish share a significantly high degree of homology compared to other species. The secondary structure indicates that both the Box I and Box II motifs are rich in proline, with a β sheet and a coiled structure in the region, where a long helical stretch precedes the Box I motif. However, the Box II motif has a coiled structure with a helix-sheet motif in all species except mouse, turkey, frog, Zebrafish, and medaka fish.

Table 3. Box I and Box II motif sequences.

Box I—Multiple Sequence Alignment Using CLUSTAL 2.20	Box II—Multiple Sequence Alignment Using CLUSTAL 2.08
Turkey-KKLLWEDVPNPKNCSWAQGV 20	Turkey PS--DQDDAFTDGGSPERGLCYLG 22
Chick-KKLLWEDVPNPKNCSWAQGV 20	Chick PS--DQDDAFTDGGSPERGLHYLG 22
Pig-KKLFWEDVPNPKNCSWAQGL 20	Frog PL--DHEDSFLEADGLERNLYYLE 22
Sheep-KKLFWEDVPNPKNCSWAQGL 20	Monkey LL--RLEGNFPEENNDEKSIYYLG 22
Dog-KKLFWEDVPNPKNCSWAQGL 20	Bat LL--TLEGNFSEENNDEKSVYYLG 22
Cattle-KKLFWEDVPNPKNCSWAQGL 20	Mouse LL--ELEGSFPEENHREKSVCYLG 22
Monkey-KKLFWEDVPNPKNCSWAQGL 20	Rat LL--ELEGNFPEENHGEKSVYYLG 22
Human-KKLFWEDVPNPKNCSWAQGL 20	Human LL--KLEGNFPEENNDKKSIYYLG 22
Mouse-KKLFWDDVPNPKNCSWAQGL 20	Camel LL--KLEGNF----------8
Rat-KKLFWDDVPNPKNCSWAQGL 20	Cattle LL--KLEGNFPEENNNERPVYYLG 22
Bat-KKLFWDDVPNPKNCSWAQGL 20	Dog LL--KLEGNFPEENNGERSVYYLG 22
Frog-KKLFWKDVPNPKHCSWAQGV 20	Pig LM--KFEGNFPKEHNDERSVYYLG 22
Zebra-KKLMWKDVPNPNKCSWAKGM 20	Zebra TS--EPDYEASENTGLAKDLYYLE 22
Medaka-KKNLVWKHVPNPKKCSWAKGL 21	Madaka MS--EHEG-VTEQRQ-VQPLCYLQ 20

3.9. The Transmembrane Region

Amino acid residues are highly conserved at various positions within the transmembrane region. These include residues at positions 843–846, 849, 855–857, 860, 862, and 864–866. The leucine at position 856 is extensively conserved across species and shows greater similarity among humans, monkeys, mice, and rats, as well as between medaka and zebrafish. Interestingly, we cannot identify any transmembrane region in camel, horse, goat, or elephant. This region contains conserved leucine (L), isoleucine, and serine (S) residues, except in medaka and zebrafish. A noteworthy observation is that tyrosine and cysteine residues are highly conserved across all species. However, in some cases, the amino acid has been replaced—for example, histidine for tyrosine in turkey, and cysteine for serine in mice and rats. These substitutions may further emphasise the vital role of the receptor dimerisation domain residues in signal transduction.

3.10. Phylogenetic Analysis

The phylogenetic analysis suggests a common ancestral gene that may have diverged into three groups at different times (Figure 3). Group 1 includes two branches, comprising pigs and sheep. Group 2 splits into two branches: the first contains humans and monkeys, while the second includes camels, horses, goats, dogs, and notably, bats. Lastly, group 3 consists of three branches: the mouse, the rat, and aquatic organisms such as fish, along with bird species like turkey, chick, and elephant. A prominent feature of the phylogram is the distinct separation between the elephant and the human. Consequently, OB-R sequences from aquatic species resemble those of bird species and rodents (mice and rats). Conversely, OB-Rb from humans and non-human primates exhibits unique sequence features, likely indicating lineage-specific divergence after the common ancestor, rather than an evolutionary trend towards increased complexity. These results highlight different evolutionary constraints and functional specializations among vertebrate lineages, rather than a strict chronological sequence of evolution.

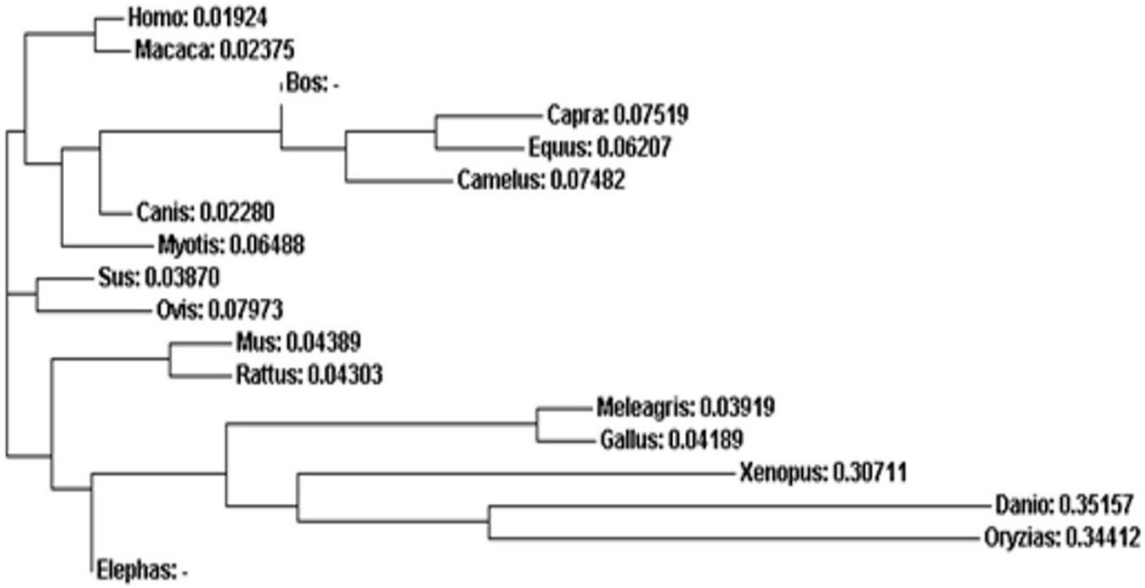

Figure 3. The phylogenetic analysis. It indicates that the OB-Rb of humans and monkeys can perform more complex functions than rodents, birds, and aquatic animals that may have evolved earlier.

4. Discussion

The structures of leptin and its receptors are conserved across diverse organisms, including humans, mice, horses, dogs, and fish [3]. However, the functional significance of this conservation across different species remains unclear. Furthermore, the leptin receptor exists in multiple isoforms, including short, soluble, and long forms, which are expressed in various tissues [4]. Therefore, the structure and function of the long-form leptin receptor, along with its evolutionary relationships among the different leptin receptor forms, are inferred from available structural data. Leptin exerts pleiotropic effects, influencing various physiological and pathological processes [5–7]. The diversity of leptin action is believed to reflect differential binding of leptin to its various receptor forms located in the plasma membrane [15–17]. The JAK2 and STAT proteins, especially STAT3 and STAT5, are essential for leptin receptor signalling and are highly conserved across vertebrates [18]. Six isoforms of leptin receptors have been generated in mammals through alternative splicing of transcripts from a single leptin receptor gene [19–21]. To analyse the evolutionary relationships of the leptin receptor from various species, including mammals, birds, reptiles, and fish, data are collected. The sequence homology results indicate that the extracellular homology is generally high among the selected species. The highest degree of homology is observed between humans, monkeys, and elephants, with closely related species showing higher similarity.

We analysed the multiple-sequence homology of leptin receptors to understand their function and evolutionary relationships. The results emphasise that the amino acid sequence in the selected species is highly conserved, particularly in the extracellular region, with increased homology. We also found that sequence similarity is greater among closely related species, especially between humans and monkeys. This similarity results from the strong connection of four cysteines in the extracellular domain of OB-Rb. Among the species analysed, serine appears more frequently than leucine, valine, and glutamic acid. The presence of leucine confers hydrophobicity to the leptin receptor. The N-terminal region of the peptide sequence begins with methionine in all species except bats. Additionally, the amino acid proline is highly conserved across all species and is structurally essential for the folding properties of the peptide.

In disulfide bond analysis, both humans and cattle show similar patterns of disulfide bridges, with cysteine highly abundant in the conserved region. The disulfide bonds mainly maintain the structural integrity of the receptor-binding sites rather than supporting the receptor's conformational stability. The secondary structure alignment uncovers standard features across species, including random coils, β-sheets, and α-helices. The increased presence of β-sheets in the N-terminal domain supports a fibronectin-like structure, similar to that of cytokine receptors. This structure plays a vital role in the formation of ligand-receptor complexes. The hydropathy plots suggest that the protein can interact with solvent molecules and show a trend in the organisation of transit peptides. The extracellular part of the human LR contains seven structural domains, with the BOX I and BOX II

motifs being more conserved. Phylogenetic data indicate that OB-Rb in humans and monkeys may have evolved later than in rodents, birds, and aquatic animals, which are believed to have evolved earlier. Although CRH2 is experimentally recognised as the main leptin-binding domain, the current study does not include explicit ligand–receptor docking or binding energy analyses. Therefore, any discussion of therapeutic targeting should be regarded as preliminary and hypothesis-generating

5. Conclusions

This research highlights that the leptin receptor, primarily the CRH2 domain, is highly conserved across species, reflecting its crucial role in leptin signalling. Mutational analysis indicates that substituting this residue causes only minor structural changes, supporting the stability and rigidity of the CRH2 core. Comparative sequence and disulfide bond analyses further underline strong extracellular homology and consistent structural features among vertebrates. These conserved patterns suggest that the fundamental mechanisms of leptin action have remained evolutionarily stable. Given the increasing global burden of obesity, metabolic dysfunction, and leptin-resistance disorders, studying these conserved domains is especially important for developing therapies. Such structural insights can help in designing targeted leptin-based modulators or biologics with better receptor specificity. Overall, this work provides a structural basis that supports future therapeutic strategies aimed at modulating leptin receptor function.

Author Contributions

R.M.: conceptualisation, methodology, software, data curation, writing—original draft preparation; N.D.: visualisation, investigation; M.R.: supervision; R.E.R.: software, methodology, validation, reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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Use of AI and AI-Assisted Technologies

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