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Natural selection and adaptive evolution of leptin

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Leptin is an adiposity-secreted hormone that is pivotal in regulating feeding behavior, energy metabolism and body mass. The study of *leptin* has been of crucial importance for public health and pharmaceutical intervention given its role in obesity. Generally, *leptin* is highly conserved due to its functional importance. However, episodes of rapid sequence evolution and positive selection have been observed in some mammalian species, indicating that the leptin functions in these animals may have undergone adaptive modification to their environments. In this article, we review the adaptive evolution of *leptin* and its potential functional consequences. This review is expected to guide future research of molecular evolution and functional assays of this gene, and also to provide a theoretical foundation for the use of leptin in therapeutic applications.

natural selection, adaptive evolution, leptin, leptin receptor

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The relationship between the adaptations of an organism to different ecological niches with genetic changes (e.g. the change of genes) has been one of the essential subjects in the study of life sciences. Accordingly, the mechanism underlying how organisms adapt to different living environments at molecular level has fascinated evolutionary biologists [1−9].

Metabolism is a series of biochemical reactions, allowing organisms to exchange materials and energy with the environment. These processes control growth, reproduction and behavior of organisms. Although the mechanisms of increased energy utilization are likely to be complex, one important component involves the activation of adipose tissue [10].

Leptin, encoded by the *obese* gene, is a hormone that mainly secreted by adipose tissue. It is important in regulating feeding behavior, energy metabolism and body mass [11−16]. The level of plasma leptin is generally regarded as a signal to direct the central nervous system (CNS) to regulate food intake and energy expenditure, which is an important pathway of metabolism to maintain constancy of the adipose mass [12,14,17,18]. The mutation of *ob* gene results in a myriad of disturbance of metabolism [12,19]. Mice that are homozygous (*ob/ob* mouse) for mutations in this gene exhibit a profound obesity resulting from defects in energy expenditure, food intake and nutrient partitioning [12,20,21]. In humans, mutation of this gene results in a profound obesity and type II diabetes [12,20−23]. Therefore, due to its critical role in obesity, the study of *leptin* will shed insight into molecular mechanism of energy homeostasis, future pharmaceutical intervention and public health [9,10,24].

The *leptin* gene, spanning over 4.5-kb, consists of three exons and two introns [9,25]. The coding exons (exons 2 and 3) are 501 bp in length in total. Leptin contains an amino-terminal signal peptide (21 residues) and a mature peptide (146 residues), with four α-helices (helices A–D) and a distorted segment E in the CD loop [9,26−28] (Figure 1).

Generally, *leptin* appears to be highly conserved due to its functional importance [12,29−32]. However, episodes of rapid sequence evolution and positive selection have been

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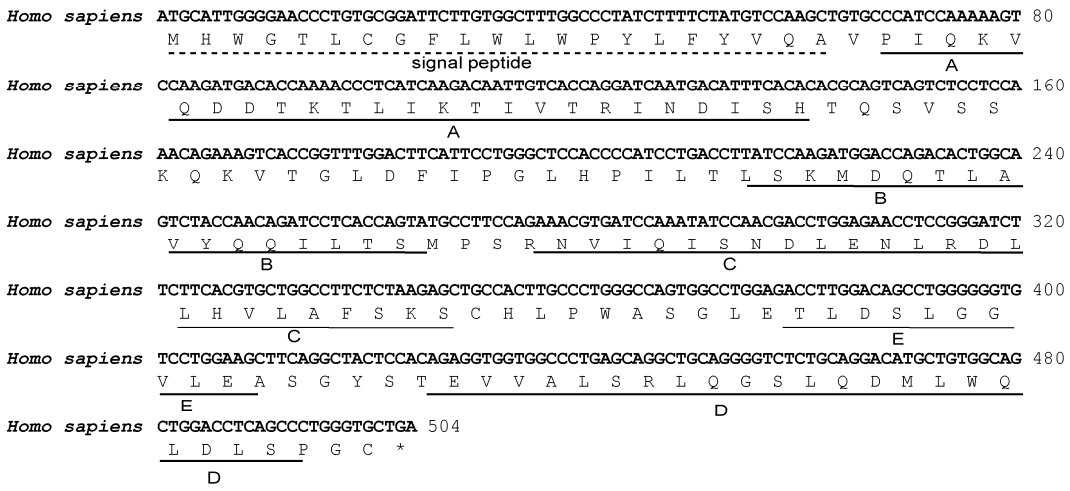

Figure 1 Human *leptin* sequence [33,40]. The signal peptide (1−21) is indicated by dotted line and the helices A−D are shown in solid lines. Helix E is a distorted segment in the CD loop.

documented in seals [9,33], whales [9], plateau pikas [34,35], primates [24,36−44] and bats [44]. The *leptin* gene duplication events were also observed in teleosts, which may be associated with tetraploidy [45]. These intriguing findings indicate that the leptin functions in these animals may have undergone adaptive modification to their environments.

In this article, we review the adaptive evolution of *leptin* and its potential functional consequences (Table 1). This review is expected to guide future research of molecular evolution and functional assays of this gene, and also to provide a theoretical foundation for the use of leptin in therapeutic applications.

1 *Leptin* evolution and high-altitude adaptation

Organisms that are living in highlands and those have been survived in the high altitude environment for millions of years have evolved various behavioral and physiological strategies to overcome the environmental challenges, which are characterized by very low temperature and hypoxia [35,46,47]. Such extreme environmental stress can drive certain proteins of these high-altitude species evolve rapidly and create a new series of metabolism reactions [35,48]. Attention of the earlier studies investigating the molecular evolutionary mechanism of adaptation to high altitude environment have been largely concentrated on mitochondrial DNA (mtDNA), which is the "energy factories" of animal cells and pivotal in oxygen consumption and energy metabolism [49]. Mt cytochrome *c* oxidase, NADH dehydrogenase and cytochrome b genes have been indicated in mammalian highland adaptation [4,49−56]. Recently, growing investigations have been performed from the perspective of nuclear genes, e.g. hemoglobin [57−60] and hypoxia inducible factor (HIF) pathway genes [61−67].

Leptin is well-known for its crucial roles in energy homeostasis. Given that changes of energy metabolism are remarkable for those species survive in cold regions at high altitudes, *leptin* thus becomes an important candidate gene for high-altitude adaptation studies. By utilizing pikas (order Lagomorpha: family Ochotonidae), which live in the Qinghai-Tibet Plateau (average altitude >3000 m), as an animal model, Yang et al. [35] examined their *leptin* gene variations. Intriguingly, they not only showed that the evolutionary rate of plateau pika leptin was accelerated, but

Table 1 Summary of the presence of positive selection of leptin on different lineages/species examined to date and potential physiological function

Order	Lineage/Species	Potential function	References
Lagomorpha	Pika	Cold adaption	[34,35]
Carnivora	Seal	Pulmonary surfactant production	[9]
Cetacea	Whale	Pulmonary surfactant production	[9]
Chiroptera	Bat	Hibernating adaption	[44]
Primates	Hominidea	Reproductive and/or dietary and/or other physiological functional adaption	[24,36–39,41,43,44]
	Gorilla	Reproductive and/or dietary and/or other physiological functional adaption	[24]
	Orangutan	Reproductive and/or dietary and/or other physiological functional adaption	[24,36–38,43]
	Macaque	Reproductive and/or dietary and/or other physiological functional adaption	[24,36–38,43]
	Mouse Lemur	Reproductive and/or dietary and/or other physiological functional adaption	[44]

also found the sign of positive selection acting on it. In total, 20 adaptive sites were identified, 9 of which located within the functionally significant segment (85−119 residues) of leptin [35,68,69]. This segment is believed to be a domain that associated with weight regulation in previous studies [68,69]. In addition, the ATP synthase α and β subunit signature site was observed only in plateau pika lineages [35]. They assume that the environment factor that drives the adaptive evolution of plateau pika *leptin* is most likely to be the extremely low temperature. Subsequently, Yang et al. [34] compared the expression levels of leptin in brown adipose tissue and the hypothalamus between plateau pika and human to test the above hypothesis. They observed that pika leptin appears superior than human leptin in inducing adaptive thermogenesis, which provides evidence that the *leptin* gene may indeed pivotal in pikas' cold adaptations associated with highland environment [34,35].

Except for humans, the Chinese snub-nosed monkeys inhabit with the highest altitudinal distribution [70−73]. In order to examine whether nature selection and adaptive evolution of *leptin* gene act on other high-altitude species, Wang et al. [74] analyzed *leptin* genes of the Chinese snub-nosed monkeys (genus *Rhinopithecus*). The Chinese snub-nosed monkeys live in mountainous regions at high altitudes (>2000 m). Similar to plateau pikas, the energy metabolism strategy of them has changed under cold conditions [4]. By sequencing *leptin* genes from two Chinese snub-nosed monkeys (*R. bieti* and *R. roxellana*), together with their lowland close relative *R. avunculus* and other Colobines, they found only 3 synonymous substitutions (*K*s) and no nonsynonymous substitutions (*K*a), indicating that the evolutionary pattern of *leptin* gene between Chinese snub-nosed monkeys and their lowland counterparts has no difference [74]. Inconsistent with Yang et al.'s [34,35] results, in which *K*a was higher than *K*s for pika *leptin* and *leptin* was considered as an important genetic factor to enhance fitness of the pikas' survival at high-altitude, Wang et al.'s [74] investigation indicates that *leptin* might not contribute much to the adaptation of high-altitude of Chinese snub-nosed monkeys [34,35,74]. In this regard, the adaptive genetic basis of *leptin*, which was detected in plateau pika, does not exist in all the high-altitude organisms, at least in Chinese snub-nosed monkeys. Other candidate genes involved in energy expenditure are more likely to contribute to the adaption to cold and hypoxia in the high-altitude environment of Chinese snub-nosed monkeys, such as hemoglobin [74,75], myoglobin [74,76], transforming growth factor-β (TGF-β) [74,77], and hypoxia-in-ducible factor1(HIF1) [74,78] as well as the mt DNA [51,79,80].

2 *Leptin* evolution and aquatic environment adaptation

Compared with the terrestrial mammals, marine mammals are a special and unique mammalian group, which have developed a series of physiological and behavioral characteristics for their adaptation to the aquatic environments [7,81]. However, investigations underlying the relationship between the genetic changes of marine mammals and their adaptation to underwater life are still limited [7,81,82]. Marine mammals have been well-known for evolving a modified and adapted fat deposit regulation and energy metabolism for their survival associated with the blubber layer [83−85]. Blubber layer is the fundamental tissue that stores energy, which is important to protect marine mammals from cold water environment [83]. Therefore, as naturally obese mammals, the marine mammals provide a good study model for the exploration of association between the leptin and the body fat regulation adaptation. Indeed, in previous physiological assays, leptin has been intensely investigated in marine mammals [86,87]. However, no evidence suggests positive correlation between the body fat mass and levels of plasma leptin in the seal and sea lion species examined so far, indicating that leptin may not mainly function in body fat regulation and energy reserves of marine mammals, but associate with other physiological process [9,33].

Hommand et al. [33] explored leptin expression pattern and the possible role of it in gray seal (*Halichoerus grypus*) and harbor seal (*Phoca vitulina*) [33]. Interestingly, they identified substantive amino acid substitutions unique in seal leptin. Furthermore, these amino acid substitutions are found to locate in the most conserved domains of helices A-D of leptin, implying that they are most likely to change the structure of leptin and have an influence on the function of this protein [26,33]. Another important finding from Hommand et al.'s [33] research is the observation of an uncommon expression of leptin in the lung tissue of seal [33]. They therefore proposed an interesting hypothesis that leptin in marine mammals plays an important role in a physiological function other than fat deposit regulation and energy metabolism, such as respiratory physiology. This hypothesis was indirectly argued for by the latter investigation of Yu et al. [9], in which *leptin* of more marine mammals, especially whales, was examined.

In Yu et al.'s [9] study, they found significant evidence of positive selection and identified several adaptive sites in toothed whales (suborder Odontoceti of Cetacea) and seals (family Phocidae of Pinnipidia), whereas no positive selection and adaptive sites were detected in baleen whales (suborder Mysticeti of Cetacea) and sea lions (family Otariidae of Pinnipedia). According to this result, Yu et al. [9] proposed that the driving forces for positive selection on toothed whales and seals leptins identified are not related to the adaptation to improve the fat deposit regulation and energy metabolism in these marine mammals because if such selection is driven solely by the difference in functions of fat deposits and the regulation of energy balance between terrestrial and marine mammals, then positive selection should occur in all the marine mammals examined.

They therefore infer that leptin is probably involved in other functions for toothed whales and seals. Previous diving behavioral studies of marine mammals showed that toothed whales and seals generally live in an underwater and deep-sea environment, whereas baleen whales and sea lions survive near the surface of the water and does not dive to great depths. Based on the diving behavior difference, Yu et al. [9] supposed that their results might reflect the increased demand for either hypoxia, pulmonary or circulatory adaptations of the deep-diving toothed whales and seals. Therefore, the extended analyses from Yu et al. [9] provide evidence supporting the hypothesis proposed by Hammond et al.'s [33] report that leptin is most likely to associate with the respiratory physiology of seals, e.g., in the respect of pulmonary surfactant production.

3 *Leptin* evolution and hibernation adaptation

Hibernation is an important strategy for organisms to tolerate food shortage and low temperature conditions [44,88−90]. During the periods of hibernation, adipose tissue plays as the fundamental source of energy helping animals to sustain life [16]. A wealthy of previous physiological studies has shown that the level of serum leptin regulated the metabolism and food intake in hibernating species, including bats, squirrels, rats, and shrews [29,91−99].

In order to determine the potential structural and biochemical differences of leptin between hibernating and non-hibernating bats, He et al. [100] sequenced the *leptin* gene of *Rhinolophus ferrumequinum* (hibernating bat) and *Rousettus leschenaultii* (non-hibernating bat), and moreover, expressed the leptin proteins from these two bats species in *Escherichia coli*. Their results demonstrated that there were more amino acid substitutions in hibernating bats leptin than in non-hibernation bats [100]. In addition, the structural modeling analysis showed that the receptor binding site III of leptin, which is critical for signal transduction, from hibernating bats, has a helical structure, whereas the same region from non-hibernating bats and human leptin was predicted to be a random loop [26,100]. The expression assay suggested that hibernating bats leptin reveals a superior inhibitory capacity for growth of 3T3-L1 cells (adipose-like cells) than that of non-hibernating bats [100]. In summary, based on the observation of the differences in amino acid sequence, protein structure, and activity between the leptins of the non-hibernating and hibernating bats, He et al. [100] proposed a hypothesis that the hibernating bats leptin may have evolved from the non-hibernating bats leptin and assumed that leptin is likely to play an important role in adipose tissue metabolism of hibernating bats in terms of satisfying the need of special energy supply during the period of torpor [100].

Subsequently, by examining *leptin* of more hibernating and non-hibernating bat species, Yuan et al. [44] interestingly revealed signature of positive selection in hibernating lineages and evidence of relaxed selection in non-hibernating lineages. In addition, their sliding window analysis revealed that in hibernating bats, most domains of *leptin* exon 3, especially in the AB loop and helix D, demonstrate higher *ka*/*ks* ratios than that of non-hibernating bats [44]. In particular, 6 of 29 residue substitutions specific to hibernating bats were found in functionally significant segment (85−119) of *leptin* exon 3, and two of them are binding sites [44,68, 69], indicating that positive selection may affect the interaction of leptin with receptor. Notably, their biochemical analysis shows that hibernating bats leptin is more efficient in lipid degradation than non-hibernating bats leptin, which probably is the biological consequence of natural selection of hibernating bats leptin [44].

4 *Leptin* evolution and other physiological functions

Accumulating evidence has suggested that besides adipose tissues, leptin was also expressed in placenta, stomach, bone marrow, lung, and mammary epithelium [17,33,101−106] and was involved in placental and fetal growth regulation, brain development, gastric function, hematopoiesis, inflammation, and pulmonary surfactant production [9,33, 101,104−107]. Accordingly, besides fat regulation and energy metabolism, it is believed that leptin may also play important roles in other physiological functions.

In view of this, it is notable that there are considerable evolutionary studies suggesting action of positive selection on the leptin of primates [24,36−44] and speculating leptin function in primates has undergone modification with new tissue specificity or some more crucial physiologic functions, although the precise ecology and selective forces acting to produce these changes are not clear. Siltberg and Liberles [43] proposed a hypothesis that the natural selection of *leptin* gene in primate maybe responding to dietary and/or reproductive variation during the evolution of primates, which will be tested in future studies.

Thus, some researchers proposed that the utility of the widely-used rodent model for studying human obesity may be limited in pharmaceutical application, as evidenced by the difference of evolutionary pattern of primates and other mammalian *leptin* genes [24,36−38]. Intriguingly, these previous studies disagreed on the specific primate lineage on which the adaptive evolution occurred. Based on different methods used for assigning ancestral sequences and calculating the *K*a/*K*s ratio [41], the exact numbers of positive selection throughout the primate lineages are not clear and from two to four lineages have been proposed (Figure 2). Benner, Trabesinger, and Schreiber [24], and Siltberg and Liberles [43] identified positive selection acting on 4 lineages, the lineages leading to hominoids, orangutans, gorillas and possibly, macaque (symbol ★ in Figure 2).

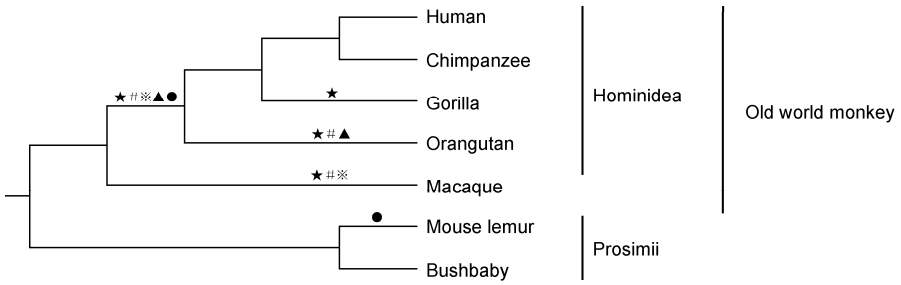

Figure 2 The primate lineages that were detected under positive selection in previous studies. ★ indicates those from Benner et al. [24] and Siltberg and Liberless [43]; # indicates those from Benner et al. [37] and Benner and Gaucher [38]; ※ indicates those from Liberles et al. [41] and Berglund et al. [39]; ▲ indicates those from Benner et al. [36]; ● indicates those from Yuan et al. [44].

Benner et al. [37] and Benner and Gaucher [38] detected positive selection on three lineages, the lineages leading to hominoids, orangutans, and macaque (symbol # in Figure 2), whereas in Liberles [41] and Berglund et al. [39], two lineages leading to hominoids and possibly, macaque, showed evidence of positive selection (symbol ※ in Figure 2). Benner et al. [36] suggested that two lineages leading to hominoids and orangutans, but not to hominoids and macaque, have undergone positive selection (symbol ▲ in Figure 2). Yuan et al. [44], however, provided evidence indicating that the lineages leading to hominoids and mouse lemur (*Microcebus murinus*, Prosimii) have undergone adaptive evolution (symbol ● in Figure 2). Hence, identification of the precise patterns of *leptin* gene evolution in primates has been elusive.

It should be noted that in all the previous evolutionary studies of primate *leptin*, the sequences used were very limited, making current understanding of primate *leptin* gene evolution largely based on the analyses of very limited taxonomic sampling. Sampling additional primate taxa will be useful for assisting in discriminating among these alternative hypotheses, which is critical for learning the true evolutionary pattern of primate leptin and its subsequent biological outcomes.

5 *Leptin* and *leptin receptor* co-evolution

Leptin is thought to exert its profound physiological effects via direct binding to the leptin receptor (LPR) in the hypothalamus [13,17,21]. The leptin receptor is a large single-transmembrane-domain receptor of the class I cytokine receptor family and is coded by the diabetes (*db*) gene [10,108−111]. Homozygous mutant *db*/*db* mice produce a syndrome that is phenotypically identical to the *ob*/*ob* mouse [108,109,112,113]. In humans, the *LPR* gene consists of 20 exons, spanning over 70 kb in chromosome 1q31 and encoding a huge protein of 1165 amino acids [10,111,114], which consist of a 22-amino-acids signal peptide and a huge mature peptide of 1143 amino acids, which contains three regions — the extracellular region (816 residues), the transmenmbrane region (23 residues) and the intracellular region (304 residues) (Figure 3) [10,111,115]. The extracellular region contains two cytokine receptor homology domains, i.e. cytokine receptor homology domain 1 (CRH1) and cytokine receptor homology domain 2 (CRH2) [9,110,115,116]. Based on previously studies, the CRH2 domain is essential for the interaction of leptin and its receptor [9,115,117,118], and it bind to the site II in leptin [9,110,115−118].

In contrast to other cytokines and their receptors, e.g., growth hormone (GH) and the growth hormone receptor (GHR) [119,120], and prolactin (PRL) and the prolactin receptor (PRLR) [121], the evolutionary pattern of leptin/receptor signaling system is still largely unknown. Some cytokines and their receptors have been shown to evolve in a coordinated manner [121], which is consistent with the ligand-receptor co-evolution hypothesis. It is interesting to know whether the *LPR* shows a similar pattern of episodic evolution to that of leptin gene. So far, the only two evolutionary analyses of the *LPR* are performed by Benner et al. [24] and Yu et al. [9], respectively. In Benner et al.'s [24] analyses of the extracellular region of primates *LPR*, although they predicted that the leptin receptor may have responded to selection in the primates, similar to that of leptin, however, they could not provide evidence of positive selection for *LPR* and co-evolution of leptin and its receptor, because fewer mammalian *LPR* genes were available for analysis than the corresponding *leptin* genes. In particular, only one *LPR* sequence from human is included in the primate lineage of their study. In Yu et al.'s [9] study, they analyzed the representatives of marine mammals — Cetacea and Pinnipedia. Their study found no evidence of positive selection for *LPR* across Cetacea and Pinnipedia lineages, which is a case contrasting to leptin of the two lineages, for which adaptive evolution has been indicated. Hence, the co-evolution of ligand-receptor pair is not observed at leptin and its receptor genes of the two marine mammalian lineages. The authors suggested that the different evolutionary patterns of *leptin* and *LPR* observed here may reflect multifunctionality of leptin and leptin receptor, and support the conclusion that the biological role of leptin varied from species to species [38]. In furture investigations, including more *LPR* sequences from other mammalian species would

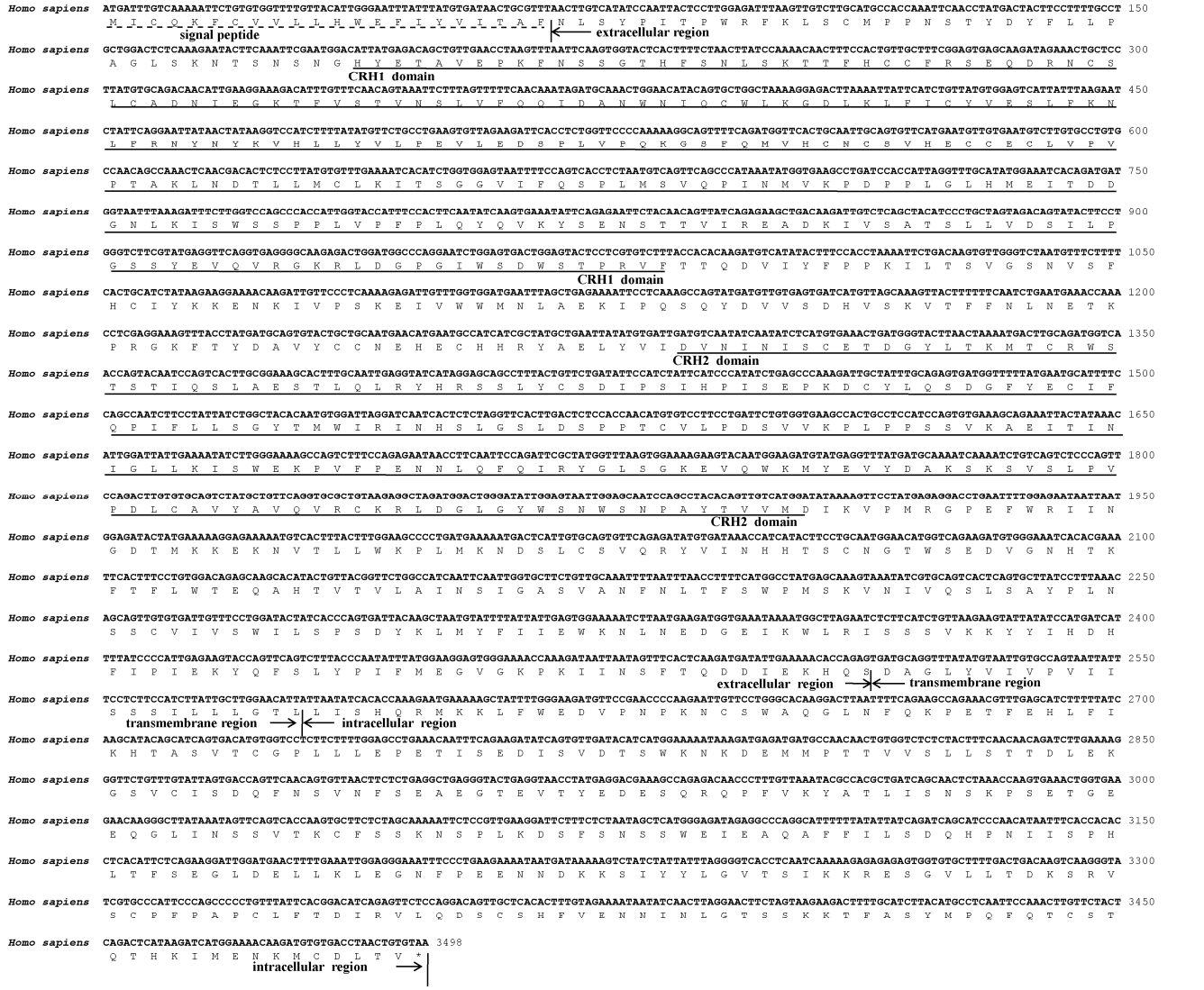

Figure 3 Human *LPR* sequence [10,110,111,115,116]. The signal peptide (1−22) is indicated by dotted line and the extracelluar region (23−838), the transmenmbrane region (839−861) and the intracellular region (862−1165) are shown between arrows. The CRH1 domain and CRH2 domain are indicated in solid lines.

give a clearer and more complete picture of the co-evolution of *leptin* and *LPR*.

6 Conclusion and perspectives

As an essential hormone for regulation of appetite and body mass in mammals, the physiological significance and medical importance of leptin and its receptor gene assures that this hormone-receptor complex will remain a focus of future studies. Previous studies have made significant progress in the evolutionary history of *leptin*, revealing the evidence for episodic evolution of this gene in seals, whales, pikas, primates, and bats etc. (summarized in Table 1). More information on the structure, function, and evolution of *leptin*/receptor genes in mammals is required for resolving the puzzle why episodic evolution occurs in certain lineages of the mammalian lineages, and not other lineages. In addition, previous studies have established a foundation for further experimental investigations. It will be interesting to test expression patterns, binding affinities and specificity of the leptin and the receptor, as well as the functional effects of positively selected amino acid changes identified so far. These findings emphasize on a need to study *leptin* and *LPR* genes in additional mammal species that encompass varying life histories. As more mammalian species are sequenced and analyzed, additional cases of demonstrating dynamic evolution would be expected to be observed for *leptin* and its receptor.

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