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NHLH2: At the intersection of obesity and fertility

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Abstract

Nescient helix loop helix 2 (NSCL2/NHLH2) is a neuronal transcription factor originally thought to be involved in neuronal development and childhood neuroblastomas. Accumulating evidence has since identified roles for NHLH2 in adult phenotypes of obesity and fertility. Here, we summarize these findings, and attempt to link genotype with phenotype in mouse models and humans. In particular, NHLH2 (*Nhlh2* in mice) is one of only two genes that are genetically linked to physical activity levels. *Nhlh2* also controls obesity and fertility, with strong sexual dimorphism displayed for both phenotypes by *Nhlh2* mutant animals. We propose that *Nhlh2* might function as a molecular sensor in different adult hypothalamic neurons to regulate energy balance, leading to normal body weight and reproduction.

Keywords

reproduction; exercise; body weight; sexual behavior; transcription

Nhlh2 during development: lessons from the knockout mouse

It has been over 20 years since the cloning and expression analysis of NHLH2 (*Nhlh2*-mouse); also called NSCL-2 and Hen2) from a murine 11.5 embryo library [1]. Due to its embryonic expression pattern, (and apparent lack of adult expression, owing to less-sensitive Northern analysis of whole brain homogenates and cell lines at that time), *Nhlh2* was originally thought to be primarily involved in neuronal development and childhood neuroblastomas [1, 2]. However, the creation of *Nhlh2* knockout (N2KO) mice identified *Nhlh2* as a mediator of adult body weight control and fertility [3]. N2KO mice were the first mouse model where deletion of a neuronal transcription factor resulted in adult-onset obesity. Both genders of N2KO mice also displayed hypogonadism, accompanied by reduced or absent sexual behavior and fertility [3, 4]. The phenotype of the N2KO mouse suggested either that embryonic expression of *Nhlh2* led to these adult phenotypes without embryonic lethality (i.e. due to compensation by another gene), or that *Nhlh2* was expressed in the adult central nervous system (CNS) where it might control neuroendocrine processes. Indeed, *Nhlh2* expression is found throughout the developing CNS and peripheral nervous system (PNS), starting at around day 9.5–10 [3], [5], but there also may be compensation/complementation by *Nhlh1*, a related bHLH protein (Figure 1).

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Nhlh2 coordinates with Nhlh1

Nhlh2 shares a strong homology to Nhlh1 (also named NSCL-1 and Hen1), with only three conservative amino acid changes in the C-terminal region containing the DNA binding HLH domain, essentially forming a small sub-family within the larger group of neuronal bHLH transcription factors [1, 6] (Figure 1). In addition, both genes are expressed in largely overlapping patterns in different areas of the CNS and PNS, during embryonic development, suggesting potential redundant functions [7, 8]. In contrast to Nhlh2 mice, Nhlh1 mutants develop normally, are fertile and show no apparent morphological abnormality [7, 9], although a decreased life span of Nhlh1 mutant mice was reported, which was ascribed to a dysfunction of the autonomic nervous system, including heart arrhythmias [9]. Generation of Nhlh1/2 compound mutant animals revealed a virtually complete absence of GnRH-1 neurons in posterior parts of the brain at E18.5, confirming overlapping functions of Nhlh1 and Nhlh2 for the generation of specific neuronal cell populations [10]. Moreover, the combined inactivation of Nhlh1 and Nhlh2 led to a complete absence of the pontine nuclei, involved in motor activity, and a strong reduction in reticulotegmental nuclei, an area within the floor of the midbrain that affects the cerebellum and its axonal projections [11]. It seems likely that conditional inactivation of Nhlh2 in Nhlh1 mutants will reveal additional overlapping functions at sites with strong co-expression of both genes, such as the olfactory epithelium and the cerebellum. Taken together, the available data results suggest that Nhlh2 might act as an important morphogenetic factor, although the latter function is largely masked by the compensatory action of Nhlh1.

Nhlh2's downstream regulatory targets

In the adult mouse, several studies, and the Allen Brain atlas show expression of Nhlh2 in the adult hypothalamus, thalamus, hippocampus, habenula, olfactory and retinal epithelium, and both fore- and hindbrain structures [5, 12]. Highly differentiated adult neurons, including pro-opiomelanocortin (POMC), thyrotropin releasing hormone (TRH), melanocortin 4-receptor (MC4R), and gonadotropin hormone releasing hormone (GnRH) neurons, express Nhlh2 [10, 12, 13], which in turn regulates a specific group of target genes in these neurons.

As a class II member of the basic helix-loop-helix (bHLH) family of transcription factors, it was expected that Nhlh2 would form dimers mainly with class I members, such as the ubiquitous bHLH proteins E12 and E47, and bind to Enhancer Box (E-box) motifs, prototypical bHLH binding sites. However, a promoter that utilizes the Nhlh2:E47 complex has not yet been identified. On the other hand, Nhlh2 heterodimerizes with E12, and a lim homeodomain protein involved in tissue patterning and differentiation, to regulate expression of *neccin*, a growth suppressor gene that facilitates the entry of the cell into cell cycle arrest [10]. For Nhlh2-mediated control of the *neccin* gene in GnRH neurons, Nhlh2 can partner with either the lim homeodomain proteins LMO-2 or LMO-4, or the class I bHLH factor, E12 [10]. In addition, Nhlh2 can partner with the leptin-regulated transcription factor Signal transducer and activator-3 (Stat3) in the regulation of *prohormone convertase 1/3* (*Pcsk1/3*), an enzyme that cleaves neuropeptides. *Pcsk1/3* gene regulation in response to leptin or food intake requires an Nhlh2:Stat3 heterodimer for full gene expression. Consequently, N2KO mice have significantly lower levels of *Pcsk1/3* than wildtype (WT) mice. This in turn results in lower levels of *Pcsk1/3* processed neuropeptides, including mature POMC and TRH neuropeptides [12]. The interaction of Nhlh2 with Stat3 might be of particular importance for the regulation of obesity and infertility, since disruption of Stat3 in neurons also causes obesity and infertility, partially recapitulating the Nhlh2 phenotype [14].

Nhlh2 can therefore interact with several different non-bHLH factors to regulate genes involved in obesity and fertility control. With the exception of the related bHLH transcription factor SCL/tal-1, the ability to interact with leucine zipper transcription factors, such as Stat3 has not been demonstrated for other Clade A bHLH family members. At this time it is not clear whether the interaction between the zinc finger in LMO-2 or LMO-4, or with the leucine zipper on Stat3 require the Nhlh2 bHLH domain or some other motif on the protein. Structural analysis of tal1's interaction with LMO2 reveals that a tal1:E47 heterodimer interacts with LMO2, which then forms a bridge interaction with the transcription factor GATA 1 in an intramolecular complex [15]. As Nhlh2 and tal1 share approximately 63% homology over their bHLH domains [1], it is possible that a similar Nhlh2:E47:LIM2 or Nhlh2:E47:stat3 complex is formed in neurons.

Nhlh2's gene regulatory targets are still being discovered. Microarray analysis of hypothalamic RNA of WT and N2KO mice, collected under different experimental energy (food intake) conditions, identified possible Nhlh2 regulated gene networks. In this analysis, 7,492 genes were differentially-expressed between WT and N2KO mice, and of these, many were overexpressed in N2KO mice [16]. These data suggest that Nhlh2 may act as a transcriptional repressor directly or indirectly, by regulating genes that repress transcription. Indeed, the promoter encoding monoamine oxidase-A (MAO-A) is transcriptionally repressed by Nhlh2 [17]. MAO-A, which degrades neurotransmitters such as serotonin and dopamine, is elevated in humans with clinical depression, and MAO inhibitors are commonly used in treatment regimens [18]. Repression of MAO-A by Nhlh2 suggests that N2KO mice might express MAO-A at increased levels, and consequently, display depressed behavior. Although this has not been tested directly, as will be discussed below, N2KO mice do show reduced physical activity and lower overall home cage activity, which might result from a depression-like state. It is likely that other Nhlh2 gene regulatory targets identified by microarray analysis, and other methods will be found to be responsible for the phenotypic deficits found in the N2KO mice.

Regulation upstream of Nhlh2

Both the Nhlh2 gene and its protein product are subject to regulation. Food intake following food deprivation, results in increased hypothalamic Nhlh2 expression [13]. Food intake results also in sharply increased circulating leptin levels and intraperitoneal leptin injection into fasted animals results in nearly a 100X increase in hypothalamic Nhlh2 levels [13], linking circulating leptin levels to high Nhlh2 expression. Leptin binds to the leptin receptor (LEPR-B) resulting in activation, and nuclear translocation of the Stat3 transcription factor [19]. As the Nhlh2 promoter contains at least 5 putative Stat3 sites proximal to the transcription start site (NC_000069.5), they could serve to mediate Stat3 binding and leptin responsiveness. Glucose, fatty acids or other circulating molecules that increase with food intake could also act to stimulate pathways leading to Nhlh2 expression, and are currently being examined. The Nhlh2 protein is also the target of post-translational modifications, namely acetylation, which inhibits its transcriptional activity [17]. Along these lines, it was recently shown that SIRT1 deacetylates Nhlh2 (discussed in more detail below) and deacetylated Nhlh2 has increased ability to activate MAO-A. Also, using the protein secondary modification prediction software ProSite, it has been suggested that Nhlh2 protein, similar to other related bHLH transcription factors, such as tal-1, may be subject not only to acetylation but also to phosphorylation. Finally, another putative post-translational modification of Nhlh2 is N-myristoylation [20]. Overall, it appears that fine-tuning of the level of Nhlh2 mRNA and active Nhlh2 protein occurs by several mechanisms that might be specific to both the external signal and the neuronal cell type receiving it.

Nhlh2 and physical activity

The balance between food intake and energy expenditure is a key determinant of body weight. Animal models of obesity predominately show increased food intake accompanied by reduced energy expenditure, either through physical activity, metabolism, or both [21]. N2KO mice were one of the initial mouse models of obesity that did not exhibit hyperphagia, or increased food intake prior to the onset of obesity, but had low levels of spontaneous physical activity [22]. Indeed, N2KO mice show very low levels of voluntary wheel running activity. While no difference in the circadian pattern of running was found, and the majority of activity for all genotypes (WT, heterozygous (HET) and N2KO) and for both sexes occurred in the dark phase, overall activity levels are reduced by at least 60% [22, 23]. N2KO mice do not appear to have gross motor or balance defects, as they can run for up to 5 minutes on a rotorod apparatus, similar to WT mice [22]. However, while the 24-hour home cage activity is lower for both male and female N2KO mice [23], N2KO mice eat the same amount of food as WT mice, both in ad libitum conditions, and refeeding following food deprivation [23]. Collectively, these results confirm that N2KO mice have low overall activity levels, accompanied by unchanged food intake. Analysis of possible metabolic changes in N2KO mice are ongoing, but regardless, these animals allow for further study of the causes and consequences of low or unmotivated physical activity, in the absence of increased food intake.

Studies in humans and animal models have found that physical activity levels have a genetic component with approximately 60% heritability, and a significant environmental component [24] as well. The first study to identify quantitative trait loci (QTL) for physical activity levels in strains classified as “couch potatoes” (low activity levels, C3H/HeJ strain), versus the highly active strain (C57L/J), found four QTL linked to running distance, duration and/or speed [24]. However, this study was only able to account for 11–34% of the genetic contribution of physical activity and did not identify a locus near Nhlh2. A subsequent study using epistatic analysis of QTL to examine genetic factors that interact to control physical activity levels identified two loci near Nhlh2 as important for the genetic contribution to duration and distance of running wheel behavior [25]. In fact, the genetic contribution of only two genes, dopamine receptor 1, and Nhlh2 meet the accepted criteria of physical activity candidate genes, in having at least three independent lines of evidence linking the gene to voluntary activity level [24].

The product of the POMC gene is a pro-neuropeptide that is cleaved by PC1/3, and the related convertase PC2, to produce several active neuropeptides, including adrenocorticotropin hormone (ACTH), β -endorphin, and α -melanocyte stimulating hormone (α MSH). Since Nhlh2 controls mRNA expression of the PC1/3 gene, the activity of Nhlh2 therefore controls the level of all active neuropeptides produced from the POMC pro-neuropeptide. α MSH and ACTH levels are clearly reduced in N2KO mice [12]. However, it was proposed that reduced physical activity levels could be due to the reduction in a β -endorphin-induced “runners high” [23]. Without the runners high, N2KO mice might have no motivation for continued voluntary or forced physical activity. If true, then POMC-specific or more specifically a mutant in which only β -endorphin were reduced by the deletion of Nhlh2, should also result in low physical activity levels. Deletion of Nhlh2 in POMC neurons, and then adding back specific POMC neuropeptides, will help us test which one is key to activity levels, and provide us with a better understanding of how Nhlh2 activity controls the motivation and/or ability for physical activity through POMC. Alternatively, Nhlh2 could play a role in PVN-mediated autonomic control of physical activity, which is also testable.

Sex and Nhlh2

Puberty and sexual maturation depends on the re-emergence of GnRH secretion and requires acquisition of sufficient body energy reserves, which is sensed by key metabolic hormones such as leptin and ghrelin [26]. GnRH-neurons release the GnRH1 decapeptide in a pulsatile manner into the hypophyseal blood system via axons projecting to the median eminence (reviewed by Yin and Gore [7]). As described, previously, *Nhlh2* mutants and to an even stronger extent *Nhlh1/Nhlh2* compound mutants show a severe reduction of GnRH neurons, which are generated in the olfactory placode and migrate to the preoptic area and the arcuate nucleus in the posterior part of the brain [7]. The reduction of GnRH-neurons in the preoptic area and the arcuate nucleus that project to the median eminence might explain the hypogonadism and infertility in these mice [10]. Recent work using a genetic cell tracing approach uncovered that absence of *Nhlh2* in GnRH neurons leads to precocious termination of migration but does not affect survival of *Nhlh2* deficient GnRH-derived cells. The majority of *Nhlh2*-mutant, GnRH-derived cells that are stuck in aberrant locations switch off GnRH expression and become located to septal areas, which explains the reduction of axonal projections to the median eminence [10]. Most likely, maintenance of GnRH expression and correct axonal projections depend critically on acquisition of correct anatomical locations of GnRH neurons in the medial preoptic area and the arcuate nucleus.

It has long been noted that energy reserves are instrumental for the onset of puberty in mammals [27]. The necessity of a coupling between nutritional status and onset of puberty is particularly evident in females, which need considerable energy reserves to successfully maintain pregnancy and nurse offspring [8]. On the other hand, the negative effect of obesity upon fecundity is well established and was already described by Hippocrates nearly 2500 year ago. Obese women, in particular those with central obesity, are less likely to conceive per cycle, often show perturbations to the hypothalamic–pituitary–ovarian axis, menstrual cycle disturbance and are up to three times more likely to suffer oligo/anovulation [8]. Furthermore, obese women are more likely to lose a pregnancy, and elevated miscarriage rates are seen following natural conception, ovulation induction and assisted conception [28]. However, the mechanism which links obesity to reduced fertility remains to be fully elucidated (reviewed by Brewer and Balen, [8]).

Nhlh2-The link between obesity and infertility?

The link between fertility and obesity is evident in several mouse models. Defective leptin signaling due to mutations in the leptin gene, *obese (ob/ob)*, or mutations in the leptin receptor in mice, *diabetes (db/db)*, and in rats, *fatty (fa/fa)*, causes disruption of energy balance resulting in obesity and infertility [29]. Most phenotypic defects can be restored by neuron-specific expression of the leptin receptor (LEPR-B) transgene in (*db/db*) mice, suggesting a common leptin-dependent neuronal circuit that affects both obesity and infertility [30]. Interestingly, disruption of *Stat3* in neurons also causes obesity, diabetes and infertility [14].

The previously described *Nhlh2:Stat3* transcriptional complex, as well as the possible regulation of *Nhlh2* by *Stat2*, provides some mechanistic theories as to how disruption of *Stat3* or *Nhlh2* affects both obesity and fertility. Indeed, the phenotype of the *N2KO* mouse suggests that *Nhlh2* may mediate both the regulation of body weight, through physical activity levels, and the control of fertility, possibly through GnRH neuron migration and sex behavior motivation [3, 10, 22]. We propose that this does not occur in isolation and *Nhlh2* acts as a hypothalamic sensor of both reproductive and body weight signals. This likely occurs by (1) *Nhlh2* gene and protein regulatory changes in response to energy availability (through leptin-*Stat3*), (2) integration of the response to these signals (through *Nhlh2* target

gene regulation), and (3) resultant regulatory pathway changes leading to normal body weight and fertility (Figure 2).

Nhlh2 is likely a leptin sensor within hypothalamic neurons, and this function could explain both the hypogonadism and obesity phenotype of the mutant animals. Animals and humans with a defect in leptin or its receptor are infertile and obese [29], and Nhlh2 expression responds to leptin levels, either directly or through food intake [13]. It is possible that Nhlh2 also acts in conjunction with, or through sensing of circulating steroid hormone levels. Indeed, leptin has the potential to regulate estrogen receptor levels through Stat3 [31]. Furthermore, the estrogen receptor alpha knockout mice (E α KO) display a similar phenotype to N2KO mice, with increased body fat along with a small, but significant reduction in wheel running (estrogen-induced) and open field activity, compared to matched WT animals [32, 33]. These data suggest a link between estrogen receptor activation of steroid hormone-induced locomotor activity, lordosis and body weight, perhaps through Nhlh2. N2KO males have low testosterone levels [3], while female N2KOs have irregular cycles, appearing to be stuck in pro-estrous, suggesting a defect in estrogen secretion or response in both genders [4]. We hypothesize that Nhlh2 may itself be regulated by estrogen, or controls estrogen receptor-mediated expression of the progesterone receptor (PR) [4]; indeed, there are several E-box motifs present in the proximal PR promoter [34] which could be targets for Nhlh2 transactivation. Both of these possibilities need to be explored.

It is also possible that the link between low exercise levels, and low sexual behavior is due to overall low motivation behavior in the N2KO mice. The recent finding that SIRT1 deacetylates NHLH2 on lysine 49 thus increasing its ability to activate the target gene MAO 1 is particularly intriguing in this regard [17]. SIRT1 is a NAD⁺-dependent deacetylase that controls a number of genetic programs to adapt to changes in the nutritional status of cells and organisms. High cellular energy levels (i.e. high concentrations of NADH) repress Sirt1 activity. Transgenic overexpression of Sirt1 recapitulates several aspects of caloric restriction including increased physical activity, which has been explained by the increased need of starving animals to search food [35]. It is tempting to speculate that Sirt1 activates Nhlh2 to promote physical activity and exploratory drive, which would nicely fit to the lower voluntary running levels and low sexual behavior phenotype of Nhlh2 mutants. Control of Nhlh2 by Sirt1 might be less important for gonadal development and onset of puberty since a good nutritional status should lower the activity of Sirt1 and hence also Sirt1-dependent stimulation of Nhlh2. However, this difference could be due to adult versus embryonic requirements for Nhlh2. Indeed, Nhlh2 is required for embryonic GnRH neuron migration [10], and defects in migration has the potential to affect gonadal development and puberty, separate from disturbances in sexual behavior. Despite this conundrum, the impact of energy-sensing sirtuins on Nhlh2 regulation, as well as the interplay between estrogen, progesterone, and Nhlh2 warrants exploration and might yield further insights into the role of Nhlh2 in energy-dependent regulation of physical activity, sexual behavior and fertility.

Concluding Remarks

Over the past two decades, our understanding of Nhlh2 has gone from simply being a transcription factor involved in neuronal development to a transcriptionally and post-transcriptionally regulated protein whose actions fine-tune adult reproductive and body weight homeostasis. There are still several outstanding questions remaining (Box 2). Future studies using in vitro analysis of Nhlh2 modifications and protein-protein interactions, mouse models, including tissue and temporal specific targeted deletions, as well as the identification of more human NHLH2 SNP carriers will continue to yield new information about this small, but mighty protein.

TEXT BOX 2**SNP-ing into NHLH2**

According to the Obesity Gene Atlas, more than 1700 obesity-associated Single Nucleotide Polymorphisms (SNPs) have been identified [37]. Human NHLH2 is located at 1p13.1 with the genomic coordinates of 1:116,378,997 - 116,383,746 [1, 38] (Figure 1). There are 137 SNPs are located within NHLH2 [39]. Only one of these is a non-synonymous mutation, resulting in an Alanine to Threonine change within the coding region of NHLH2 (rs199738358), and to date, no diseases or phenotypes have been associated with this SNP. However, a nearby non-synonymous SNP [40, 41], not currently listed in the NCBI database, changes a species-conserved alanine to a proline [42]. This SNP was first found in 2 obese individuals in a small cohort [40, 41] and leads to structural changes to the protein as detected by Western and in silico analyses [42]. As Nhlh2 deletion in mice also results in obesity, it is possible that this structural change renders NHLH2 non-functional, leading to the obese phenotype in these individuals. More work is needed on this SNP, and additional carriers to make this determination.

A study recently reported on rs11805084 [42]. mRNA stability analysis showed that the SNP, within the 3' untranslated region of NHLH2, confers approximately 50% more instability to the Nhlh2 mRNA, leading to an approximately 60 minute half-life, compared to a 120 minute mRNA half-life for the species containing the wildtype 3'UTR [42].

In genomic linkage analysis studies, a locus lying just 300,000 bp from NHLH2, D1S189 has been linked to two human conditions. D1S189 is strongly linked to exercise-induced polymorphic ventricular tachycardia [43]. Humans with this condition suffer from seizures or sudden death in response to exercise or psychological stress, due to rapid, irregular heartbeats originating in the ventricles. While no instances of sudden death have occurred in Nhlh2 mice, it is possible that irregular heartbeats lead to dizziness or shortness of breath in the mutant animals, and could be responsible for lack of both voluntary and spontaneous running. D1S189 has also, more recently be linked to an autosomal dominant form of retinitis pigmentosa [44]. This degenerative eye disease can result in vision impairment and even blindness. Nhlh2 is expressed in the developing optic cup and retina [5]. Overexpression of cNSCL2 in chickens leads to retinal degeneration. It is possible that a promoter or 3'UTR SNP resulting in higher overall NHLH2 expression, or mis-expression could be responsible for the autosomal dominant form of retinitis pigmentosa linked to D1S189. In summary, there are multiple SNPs to be examined, and possible linkages to be made between NHLH2 and several human conditions.

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TEXT BOX 1**Sexual Dimorphism in N2KO mice**

Both the female and male N2KO mice show evidence of hypogonadism, and reduced adult sexual behavior, but the severity of the conditions is more evident in males than in females. Male N2KO mice are born with small, pale testes, with the delay in testicular growth evident prior to weaning [3]. Conversely, by 4.5 weeks of age, female N2KO mice showed no discernable difference in ovarian or oviduct size, histology or development [3].

Both male and female N2KO mice are hypogonadal as adults, with this phenotype particularly striking in the males, including male accessory glands. Male N2KO mice do not display any sexual behavior when exposed to primed females [3], and anecdotally, no male N2KO mice has ever sired a litter of pups. Testosterone levels are low in male N2KO mice.

Conversely, female N2KO mice are capable of having litters, and have a much more complex phenotype during puberty and in adulthood. First, during puberty, exposure to male urine increases growth and development of their ovaries and oviducts, with up to 50% of the N2KO females showing near-normal ovarian development [3]. In the absence of males, puberty in N2KO females is delayed with delayed vaginal opening and an increase in the number of days before the first estrous cycle occurs. Supplementation of N2KO mice with estradiol (E2) for 14 days resulted in normal appearance of first estrous, although supplementation with GnRH, which is reduced in N2KO mice, had no effect [36]. However, in adult females, priming with pregnant mare serum gonadotropin followed by human chorionic gonadotropin could only induce a strong ovulatory response in young (mean age 4 months) but not older (mean age 11 months) female N2KO mice. Likewise, there is a 50% reduction in total litters produced by N2KO mice during 9 months of continuous mating, even though the days to first litter are similar between WT and N2KO mice [4]. Females N2KO primed with E2 and progesterone also show a reduced lordosis response, regardless of age [4].

Outstanding Questions Box

- What other transcription regulatory proteins, including repressor complexes, interact with Nhlh2?
- How do secondary modifications mediate Nhlh2 function and/or protein-protein interaction?
- What role does Nhlh1 play in mediating or compensating for Nhlh2?
- What other genes are direct targets of Nhlh2?
- How is the Nhlh2 gene regulated in response to leptin, reproductive signals or other pathways? Does Stat3 and/or estrogen receptor play a role?
- Do human SNPs in NHLH2 contribute to any human obesity, physical activity or fertility phenotypes, and if so, are there ways that one can bypass NHLH2 action and alleviate these conditions in carriers?

Highlights

- Nhlh2 interacts with bHLH, Stat3, and LIM proteins to regulate diverse gene targets
- Nhlh2 knockout mice are sexual dimorphic in reproductive and body weight phenotypes
- Nhlh2 acts as a molecular sensor to coordinate energy balance and fertility control

Figure 1. Comparison of Nhlh2 and Nhlh1

A) Nhlh2 and Nhlh1 are most homologous in their C-terminus, helix-loop helix and DNA binding domain. The putative regulatory domains of each protein share few overlapping amino acid sequences.

B) Comparison of the alternate names, protein length, chromosomal position, possible protein interaction partners and phenotypes of the Nhlh1 and Nhlh2 knockout mice. While there are some similarities between the two related proteins, these are distinct genes, with distinct functions and interactions.

Figure 2. Nhlh2 as a central mediator of fertility and energy availability signals

Nhlh2 expression is stimulated by food intake, environmental temperature and leptin, and likely other as yet unconfirmed signals such as exercise exertion and estrogen, or other circulating hormones. Nhlh2 protein is subject to secondary modification and requires various protein partners to regulate genes that control fertility, body weight, exercise, sympathetic nervous system output to peripheral tissues, and tissue inflammation. Polymorphisms in NHLH2 could lead to changes in NHLH2 function or activity ultimately affecting body weight and fertility.