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Functional Analysis of Seven Genes Linked to Body Mass Index and Adiposity by Genome-Wide Association Studies: A Review

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Key Words

Body mass index · Adiposity · Genome-wide association studies · *FTO* · *NEGR1* · *TMEM18* · *ETV5* · *FLJ35779* · *LINGO2* · *SH2B1* · *GIPR*

Abstract

Genome-wide association studies (GWAS) have identified a total of about 40 single nucleotide polymorphisms (SNPs) that show significant linkage to body mass index, a widely utilised surrogate measure of adiposity. However, only 8 of these associations have been confirmed by follow-up GWAS using more sophisticated measures of adiposity (computed tomography). Among these 8, there is a SNP close to the gene *FTO* which has been the subject of considerable work to diagnose its function. The remaining 7 SNPs are adjacent to, or within, the genes *NEGR1, TMEM18, ETV5, FLJ35779, LINGO2, SH2B1* and *GIPR*, most of which are less well studied than *FTO,* particularly in the context of obesity. This article reviews the available data on the functions of these genes, including information gleaned from studies in humans and animal models. At present, we have virtually no information on the putative mechanism associating the genes *FLJ35779* and *LINGO2* to obesity. All of these genes are expressed in the brain, and for 2 of them *(SH2B1* and *GIPR)*, a direct link to the appetite regulation system is known. *SH2B1* is an enhancer of intracellular signalling in the JAK-STAT pathway, and *GIPR* is the receptor for an appetite-linked hormone (GIP) produced by the alimentary tract. *NEGR1, ETV5* and *SH2B1* all have suggested roles in neurite outgrowth, and hence SNPs adjacent to these genes may affect development of the energy balance circuitry. Although the genes have central patterns of gene expression, implying a central neuronal connection to energy balance, for at least 4 of them *(NEGR1, TMEM18, SH2B1* and *GIPR)*, there are also significant peripheral functions related to adipose tissue biology. These functions may contribute to their effects on the obese phenotype.

Introduction

The human genome project, and the subsequent development of tools allowing identification of genome-wide polymorphic variation at key variable nucleotides, has opened up a new era in the genetic analysis of complex traits [21]. This has included genome-wide association studies (GWAS) of several important disease states, such as diabetes [19, 108, 110, 115], hypertension [2, 57, 83],

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heart disease [72], cancer [25, 47, 112, 118], Parkinson's disease [106, 113], Alzheimer's disease [40] and chronic obstructive pulmonary disease [92]. In addition, GWAS have investigated traits that do not have disease associations such as height [135, 143] and age at menarche [90]. In the last 7 years, there have been at least 5 major GWAS studies, each analysing >50,000 individuals, that have aimed to associate the variation in single nucleotide polymorphisms (SNPs) to variation in body mass index (BMI). These include associations established across approximately 450,000 Caucasian subjects and around 150,000 East Asians [86, 89, 116, 136, 137]. BMI is defined as body mass (in kg), divided by height (in m) squared, and is a widely used surrogate for adiposity, although its limitations in this respect are well known [91, 100]. Nevertheless, BMI is an easily measured phenotype that lends itself to the large sample sizes necessary to achieve sufficient power in GWAS, and it is widely used in epidemiological studies and surveys of obesity prevalence.

Many of the target SNPs that have been identified have been replicated across different populations, lending support to the idea that they are functionally associated with obesity. However, GWAS based on more sophisticated analyses of body adiposity, using computed tomography (CT), only confirmed a link to either visceral or subcutaneous adiposity at 8 of these loci [29]. The nearest genes to these 8 SNPs are *NEGR1, TMEM18, ETV5, FLJ35779, LINGO2, SH2B1, FTO* and *GIPR*. We can be very confident that these 8 identified associations reflect some underlying biological association between the genetics and the obese state – presumably acting via an impact on energy balance [37]. Of these genes, the most well known and characterised is *FTO*, although despite this attention there still remains considerable uncertainty over its function and mechanism [e.g. 62, 70, 122]. The aim of this article is to review what we know about the functions of the other 7 less well-studied genes located nearest to the identified SNPs, and to speculate where possible what the functional nature of their linkage to obesity might be. By making this selection, I do not mean to imply that the other SNPs identified by GWAS are not associated to adiposity. There is a potential bias in the CT GWAS study by Fox et al. [29] because they only included individuals of European ancestry, and they only examined 2 fat stores. Moreover, the selection of SNPs here was based only on the overall analysis of pooled sexes, and it should be noted that some SNPs are known to exert sex-specific effects (e.g. rs1659258 near to the gene *THNSL2*). Finally, the sample size of the CT study [29] (n = 10,557) was necessarily much lower than in the other studies, because of the sophisticated and expensive nature of the phenotyping employed. This reduces the power to detect significant associations. Thus, only the SNPs with the greatest effect passed the hurdle of being significant in their study.

The evidence relating to each SNP will be reviewed in the sequence they appear in the human genome, starting at chromosome 1 and ending at chromosome 19. To date, no SNPs significantly associated to obesity have been identified on chromosomes 20–23 or the X/Y chromosomes in GWAS studies.

SNP	**rs2815752**
Other significant nearby SNPs	rs3101366, rs2568598
Chromosome	1p31.1
BMI link identified in	[116, 137]
Location of SNP	Intergenic
At risk allele	A
Nearest gene	Neuronal growth regulator 1 (NEGR1)
Synonyms	IgLON4, Kilon, neurotractin
Entrez gene reference	257194
Ensembl gene reference	ENSG00000172260

In 1999, a novel immunoglobulin superfamily member was identified that had three C2 domains and several potential sites for N-glycosylation [33]. Since the protein showed high sequence similarity to IgLON family members, it was called Kilon (kindred of IgLON). Western blotting showed that the expression of Kilon was brain specific (notably in the hippocampus). It was already detectable at the E16 stage, and its level slowly increased during development and was maintained into adulthood [33]. At the same time, a second group discovered the same protein in the chicken using a different strategy. They called the gene neurotractin [65]. They discovered that recombinant neurotractin promoted neurite outgrowth of chicken telencephalic neurons, implicating it as a key player in the regulation of neurite growth. The homology between Kilon and neurotractin was recognised by Bauer et al. [11], and they were subsequently renamed neuronal growth regulator 1 *(NEGR1)*. However, many papers continue to use the name Kilon, particularly in the brain development field. Since some papers use *NEGR1* and others use Kilon, I will refer to it hereafter as Kilon/NEGR1.

Kilon/NEGR1 is 1 of 4 members of the IgLON family, the others being limbic-associated membrane protein (LAMP), opioid-binding cell adhesion molecule (OBCAM) and neurotrimin (Ntm) (also called CEPU-1).

The function of Kilon/NEGR1 has been disputed since its initial description, with some suggesting it is primarily involved in cell-cell adhesion [71], while others maintain a function in neurite outgrowth and synaptogenesis [75, 109]. In situ hybridisation has been used to analyse the distribution of Kilon/NEGR1 in the developing and adult rat hippocampus, compared to the distribution of LAMP [11]. Gene expression of both genes was not responsive to hippocampal deafferentation and seizure, suggesting they do not play a role in neurite outgrowth.

Contrasting these studies, lesions in the entorhinal cortex did result in induction of Kilon/NEGR1 expression in reactive astrocytes where regenerative axon sprouting occurs [109]. In addition, it was shown that Kilon/NEGR1 attracted hippocampal axons and that astroglial Kilon/NEGR1 promoted neurite outgrowth [109]. These results suggested a function for Kilon/NEGR1 as a trans-neural growth factor for outgrowing axons, following hippocampal denervation. Since Kilon/NEGR1 was also found to be upregulated during brain development, and was particularly expressed on neurites of primary hippocampal neurons, a role in axon growth during hippocampal development is also implied. None of these suggested functions in the hippocampus, however, give a hint to its role in obesity.

Miyata et al. [75] suggested that there could be a role for Kilon/NEGR1 in synaptogenesis, since immunohistochemistry demonstrated co-localisation of Kilon/NEGR1 with vesicle-associated membrane protein 2, a synaptic marker protein. This suggestion of a role for Kilon/NEGR1 in synaptogenesis was supported by observations that in cultured neurons, Kilon/NEGR1 localised chiefly at axons and presynaptic terminals at the early culture stage [42]. In later stages of culture, however, it was observed mostly at the dendritic postsynaptic spines of mature neurons. Overexpression of Kilon/NEGR1 also had variable effects depending on the culture stage. Early in culture, it decreased the number of dendritic synapses, while in late culture, it increased them [41, 42].

The primary focus of work on Kilon/NEGR1 has been in the hippocampus, a brain area not traditionally associated with studies of energy balance. However, it is also found in other brain regions. Using western blotting, the protein was found abundantly in the olfactory bulb, cerebral cortex, diencephalon, hippocampus and cerebellum, with low expression levels in the medulla oblongata and spinal cord [74]. Interestingly, in the context of regulation of energy balance, high levels of Kilon/NEGR1 have also been observed in the arginine-vasopressin (AVP) and oxytocin (OXT) magnocellular neurons of the rat hypothalamus [75]. Kilon/NEGR1 immunoreactivity was localised mainly in the dendrites of AVP-secreting neurons (and also occasionally OXT-secreting neurons) [75]. The AVP and OXT magnocellular neurons in these nuclei display reversible structural plasticity under different conditions. Localised expression of Kilon/NEGR1 (and OBCAM), particularly in dendrites, might suggest that these molecules play an important role in rearranging dendritic connectivity of these neurons. Using an in vitro assay with cells derived from mice that had the Kilon/NEGR1 gene knocked out, it has been shown that Kilon/NEGR1 plays a key role in both cell-cell adhesion and neurite outgrowth in hypothalamic neurons [56]. AVP and OXT have both been implicated in the regulation of energy balance. Systemic leptin, for example, increased the electrical activity of OXT neurons in the supraoptic nucleus [124], and it restored gene expression (mRNA) levels of both OXT and AVP in fasted mice to non-fasted levels [121]. Peripherally administered OXT reduced food intake and reversed diet-induced obesity in both rats [9, 22] and mice [63]. OXT receptor-deficient mice develop obesity [117]. Arginine-vasopressin, on the other hand, seems to interact with Neuropeptide Y in the regulation of food intake [6]. Interestingly, the AVP- and OXT-expressing neurons in the supraoptic nucleus and paraventricular nucleus also express nesfatin-1, an anorexigenic molecule first identified in 2006 [84]. This points to a potential additional molecular route by which Kilon/NEGR1 may exert its effects on food intake via modulation of nesfatin-1. High levels of Kilon/NEGR1 have been reported in other nuclei of the hypothalamus [110], and nutritional state affected the gene expression levels of Kilon/NEGR1 in the ventromedial and arcuate nuclei and the substantia nigra/ventral tegmental area (VTA). This suggests a potential direct link to food intake and energy balance regulation [15]. Whether these effects are dependent on AVP, OXT or nesfatin-1 modulation remains unknown, since the changes are located in different hypothalamic nuclei.

Outside of the brain, Kilon/NEGR1 is highly expressed in several peripheral tissues (fig. 1). Relative to the brain, expression in white adipose tissue was 3-fold higher, while it was 5-fold higher in smooth muscle than in the brain and 6.5-fold higher in the pituitary gland than the brain (fig. 1) [13]. In contrast, gene expression was almost absent in the liver [13]. Walley et al. [129] explored differential gene expression in subcutaneous adipose tissue collected from 154 sibling pairs of subjects who were discordant for obesity status. Genome-wide transcript expression was measured using microarrays, and it was

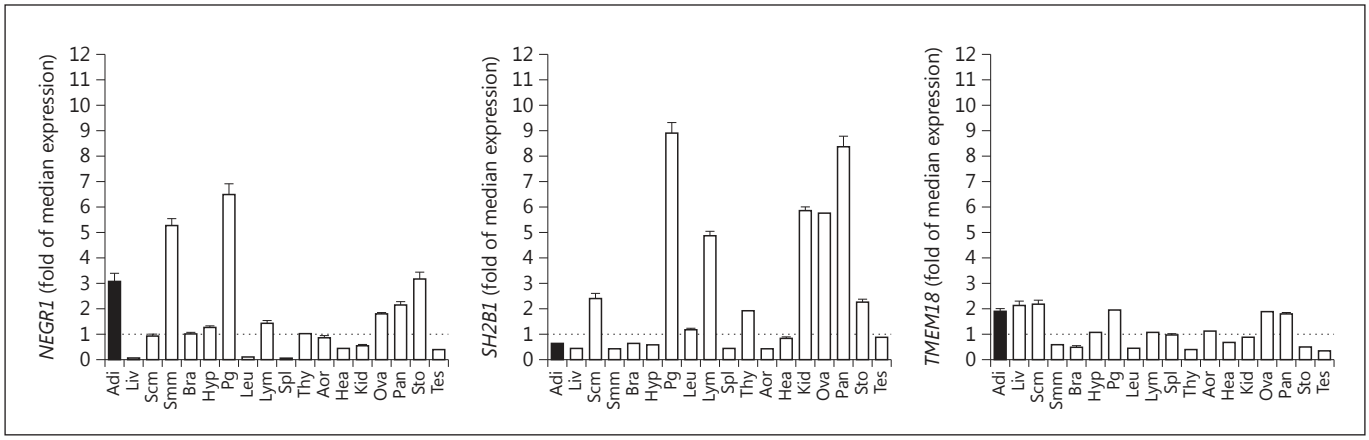

Fig. 1. Gene expression levels of 3 putative obesity genes *(NEGR1, TMEM18* and *SH2B1)* in human tissues. Adi = Adipose tissue; Liv = liver; Scm = skeletal muscle; Smm = smooth muscle; Bra = brain; Hyp = hypothalamus; Pg = pituitary; Leu = leukocytes; Lym = lymph node; Spl = spleen; Thy = thymus; Aor = aorta; Hea = heart; Kid = kidney; Ova = ovary; Pan = pancreas; Sto = stomach; Tes = testis. All 3 genes showed a wide tissue distribution despite previous suggestions that they are centrally acting [from 13].

found that almost one third of the transcripts were differentially expressed between lean and obese siblings. Cellular adhesion molecules contained the largest number of differentially expressed genes. It was noted that there was a subset of differentially regulated genes in a network, with Kilon/NEGR1 as a central hub. This suggested that, in addition to its central role as a factor influencing neuron architecture, Kilon/NEGR1 may have additional functions in adipose tissue. The expression of Kilon/NEGR1 in adipose tissue has been suggested to be linked to its role in promoting adipogenesis. In cell culture of human adipocytes, there was a 2-fold upregulation of Kilon/NEGR1 during adipogenesis [13] relative to quiescent adipocytes. A causal linkage was implied, as adipogenesis was inhibited by siRNA knockdown of Kilon/NEGR1. Insulin and IGF-1 both significantly reduced the expression of Kilon/NEGR1 in adipocytes, but it was significantly upregulated by treatment with dexamethasone. Isoprenaline had no significant effect [13].

Lee et al. [56] generated a constitutive Kilon/NEGR1-deficient mouse. Mice with the Kilon/NEGR1 gene ablated weaned (day 20) about 25% lighter than wild-type or heterozygous mice. Over the next 10 weeks, there was a clear retardation of growth, with the knockout (KO) mice being about 10% lighter at week 10, although this difference narrowed over the next 8 weeks so that by week 18, they were only 6% lighter. Interestingly, the difference in body composition between the wild-type and KO mice was solely due to the difference in lean mass rather than a difference in fat mass (fig. 2) [56]. Because the mice had equal body fat contents but different lean masses, the percentage of body fatness was elevated in the mice with the loss of the Kilon/NEGR1 gene. This pattern shows a clear difference to the situation in humans, where carriers of the at risk 'A' form of the SNP at rs2815752 (upstream of Kilon/NEGR1) have elevated levels of both subcutaneous and visceral fat stores, as determined by CT [29]. This latter observation for a role in regulating the levels of fat stores in humans is supported by the effects of Kilon/NEGR1 in human adipogenesis [13], where it was shown that Kilon/NEGR1 was 2-fold upregulated during adipogenesis, and siRNA knockdown of Kilon/NEGR1 inhibited adipogenesis [13].

The metabolic rate of the Kilon/NEGR1 KO mouse was lower than that of wild-type mice, but normalising for lean body mass completely removed this effect, implying no impact of the gene on energy expenditure [56]. Although division by lean mass was perhaps not the best way to normalise such data [7, 119], the fact that it removed the apparent impact of the gene on energy expenditure is striking (fig. 3). There were also significant effects on food intake and physical activity levels, both of which were lower in the KO mice. However, no normalisation with respect to body or lean mass was performed for these variables. In humans, a study of 1,700 Dutch females found there was an association between Kilon/NEGR1 genotype and macronutrient intake [12], pointing to a centrally mediated impact on intake, rather than expenditure.

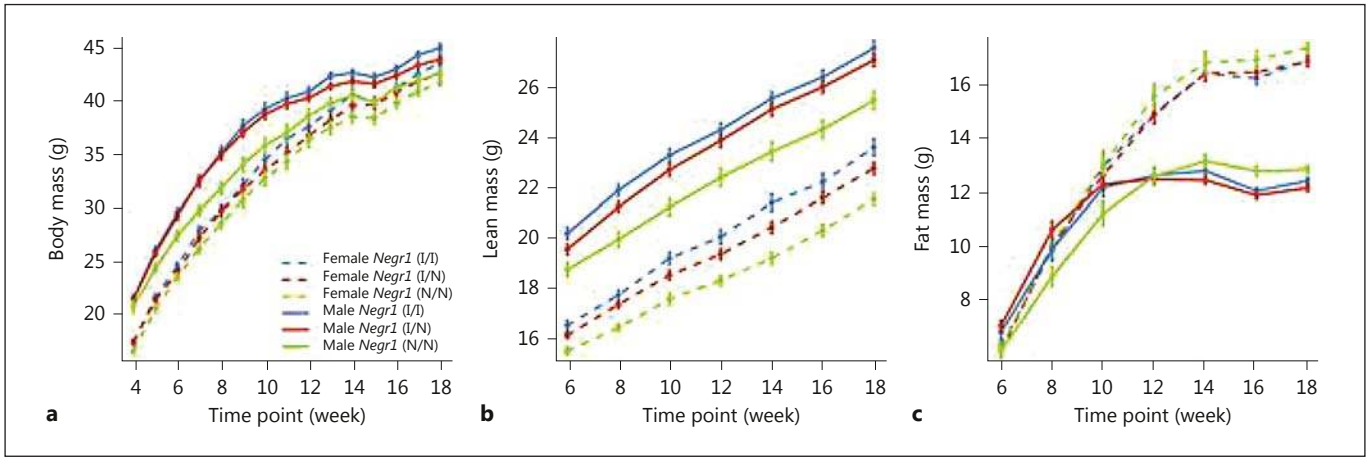

Fig. 2. Body weight (**a**), lean mass (**b**) and fat mass (**c**) gains over time in male and female mice that are wild type (I/I), heterozygous (I/N) or homozygous null (N/N) for the gene *NEGR1*. The growth curves show an effect of the gene absence on total body weight in both sexes, but this stems mostly from an impact on lean tissue mass rather than on fat mass [from 56].

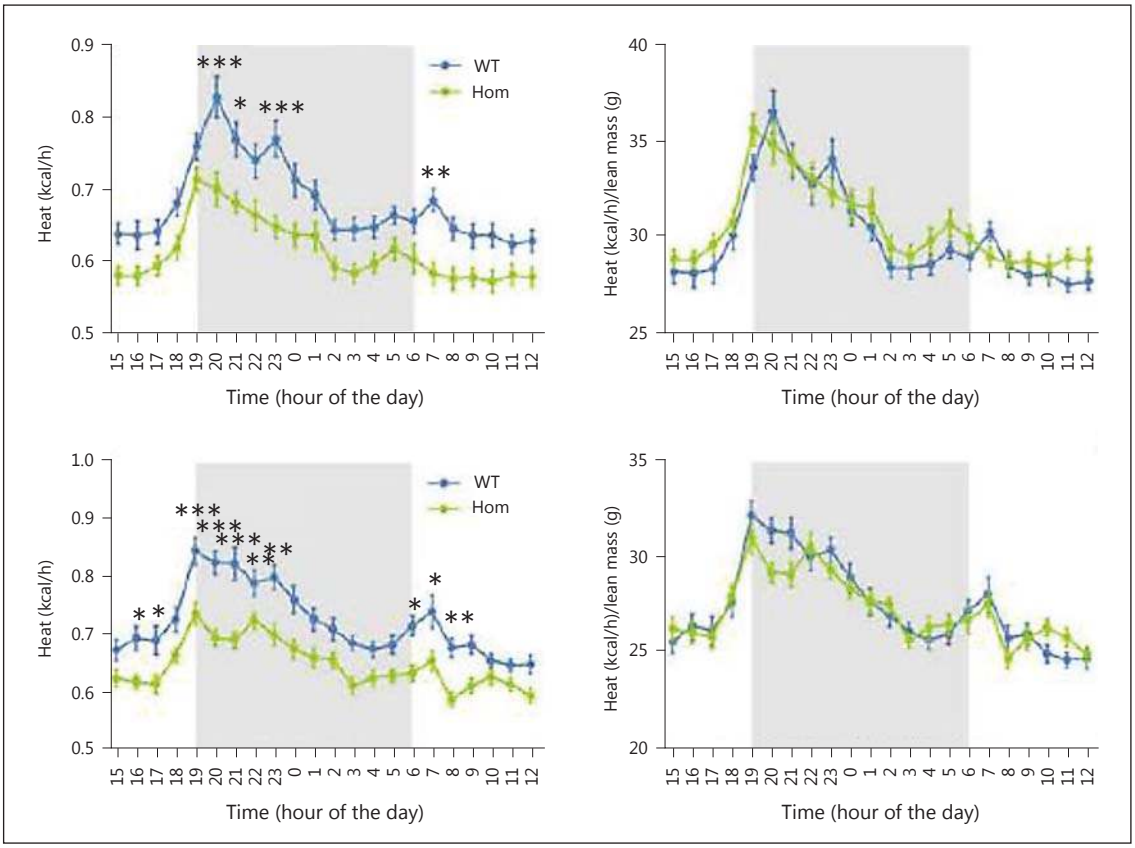

Fig. 3. Differences in energy expenditure between homozygous NEGR1–/– mice and wild-type mice measured over 24 h. Left panels show raw data (male top, female bottom) and right panels show the same data normalised by dividing by lean mass [from 56]. Normalisation removed the impact of the gene KO in both sexes, indicating that the differences in energy expenditure at the whole-animal level were entirely due to the impact of the gene on lean tissue levels. WT = Wild type; Hom = homozygous NEGR1–/– mice. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

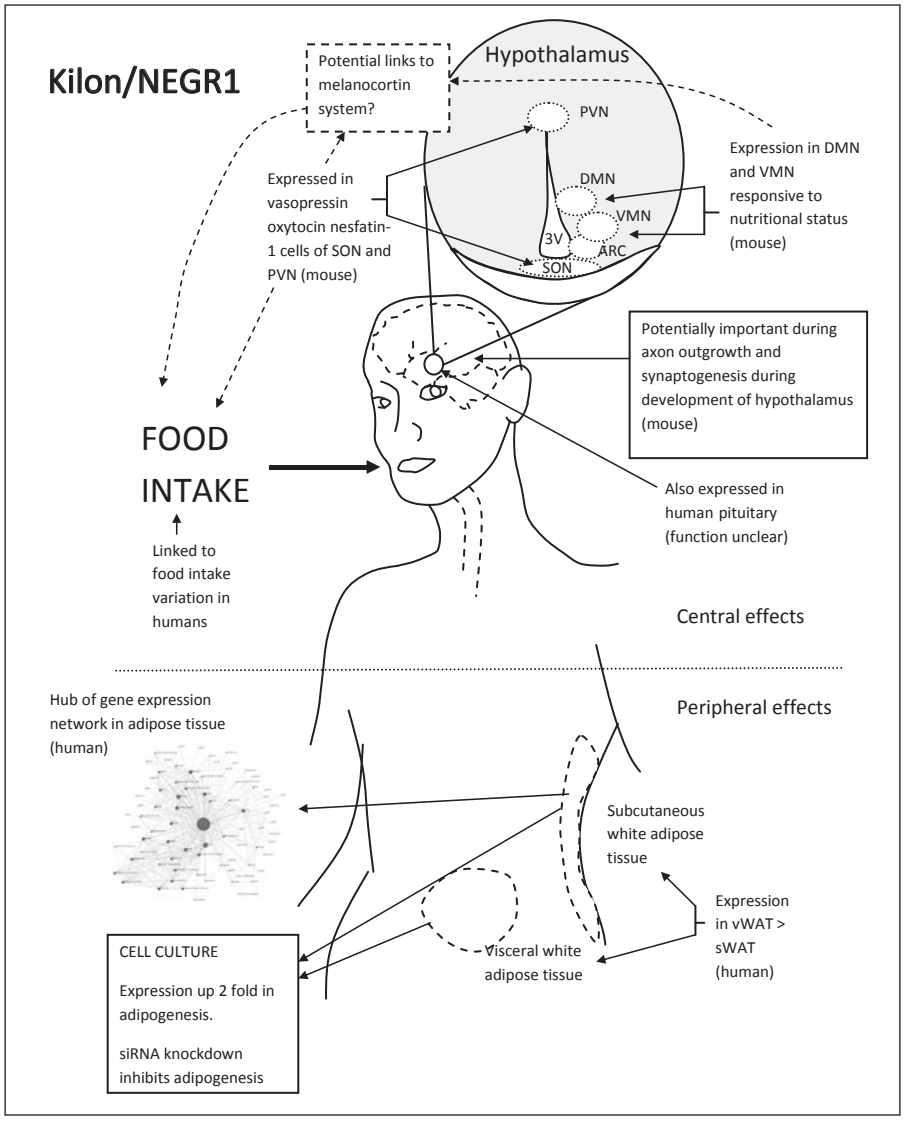

Fig. 4. Diagram summarising the effects of NEGR1 in relation to obesity.

The genetic variants upstream of Kilon/NEGR1 that are linked to BMI are also linked to insulin sensitivity (independent of their effects on BMI) and, hence, may partially mediate the link between obesity and diabetes [22]. In this context, given the different importance of subcutaneous and visceral adipose tissue for insulin resistance and type 2 diabetes risk, it is interesting that Kilon/NEGR1 gene expression was significantly lower in subcutaneous compared to visceral white adipose tissue [13].

In summary, Kilon/NEGR1 is a cell surface protein found extensively in the brain, which appears to be involved centrally in synaptogenesis, neurite outgrowth and cell-cell recognition/adhesion. Localised expression of Kilon/NEGR1, particularly in dendrites, suggests it might play an important role in rearranging dendritic connectivity. It therefore probably plays a key role in the development and plasticity of the hippocampus and hypothalamus. The responsivity of gene expression for Kilon/NEGR1 in the hypothalamus to nutritional status indicates a potential role in modulating energy balance, potentially linked to AVP, OXT and nesfatin-1 signalling. This may include effects on dendritic connectivity of neurons containing AVP and OXT receptors. This central role is supported by an association between the genetic polymorphisms upstream of Kilon/NEGR1 and food intake in humans. There is, however, a conflict in the data relating to its function in mice and humans. Mice that were null for the gene had a greater percentage of body

fatness, but this was because of an impact on lean tissue mass, not on fat tissue mass. In contrast, CT studies in humans pointed to a direct impact of the GWAS-identified SNP on fat content. Moreover, there is a suggestion that the gene may be involved directly in adipogenesis in humans. Wider and higher gene expression levels in smooth muscle and the pituitary may underpin other unknown functions related to energy balance. Future studies would benefit significantly from the creation of conditional KO mice, allowing the central and peripheral functions of the gene to be dissected. Moreover, the interactions between Kilon/NEGR1 and both OXT and AVP status could be profitably explored by examining the changes in gene expression of Kilon/NEGR1 in response to peripheral infusions of OXT and compounds that affect the AVP signalling such as Apelin [82, 107] and nesfatin-1 [53, 84, 114]. Links of Kilon/NEGR1 to nesfatin-1 status may be particularly revealing. The relationships between Kilon/NEGR1 and obesity are illustrated in a summary diagram (fig. 4).

SNP	**rs2867125**
Other significant nearby SNPs	rs2867125, rs4854344, rs7561317
Chromosome	2p25.3
BMI link identified in	[116]
Location	Intergenic
At risk allele	C
Nearest gene	Trans-membrane protein 18 (TMEM18)
Entrez gene reference	129787
Ensembl gene reference	ENSG00000151353

TMEM18 is a small 140 amino acid protein (17 kDa) that is predicted to consist mostly of α helices and has 3 transmembrane domains. Phylogenetic analysis has revealed that the gene has an ancient origin, being found in plants and most other eukaryotic organisms. It is remarkably well conserved across this 1,500 million year evolutionary history [3]. Surprisingly, it has no clear homologs in either yeast or *Caenorhabditis elegans*. It has a wide distribution of tissue gene expression in both rat and mouse (fig. 5) [3]. In the rat, outside the brain, the highest expression levels were found in skeletal muscle, heart, kidney and the reproductive organs (uterus or testicles). In the mouse, a narrower range of peripheral tissues have been screened, and the highest expression was in the kidney and lung, with relatively low levels in the heart. In human tissues, a similar wide distribution of expression has been found, with maximal levels in adipose tissue, liver and skeletal muscle [13]. Expression was lower in subcutaneous, compared to visceral adipose tissue, and lower in adipose tissue from obese relative to lean subjects [13]. In the brain, there is a widespread distribution, with particularly high expression in the rat in SL7, SL8, brainstem, cerebellum and eye. In the mouse, the highest brain expression occurred in the hypothalamus, thalamus and substantia nigra [3]. These broad patterns of expression do not support the early suggestion that expression is particularly dominant in the hypothalamus [137]. Across 5 regions of the mouse brain (cortex, amygdala, hypothalamus, thalamus and hippocampus), TMEM18 was found in 73% of neurons, and 64% of non-neuronal cell types, with no clear differences between brain regions [3].

Early studies suggested an association between the TMEM18 protein and the nuclear membrane, rather than the cell surface [3, 52]. Two groups have used protein modelling software to predict the structure of the protein, and both predict 3 transmembrane α helices [3, 42]. Both also noted that the C terminus of the protein has an array of large hydrophilic amino acids, which causes that end to extend out from the membrane. Jurvansuu and Goldman [51] indicated that the C terminus extends into the nucleus, rather than out into the cytoplasm, but Almèn et al. [3] suggested the reverse. The protruding part of the C terminus, with several positive amino acids, is a good candidate to bind DNA, particularly if it projects into the nucleus. By incubating together chromosomal DNA linked to cellulose, and protein extracts from TMEM18-overexpressing cells, proteins bound to the DNA were eluted with a high salt concentration, and these included TMEM18 [51]. However, mutated TMEM18 lacking the 13 C-terminal amino acids did not bind to either single or double-stranded DNA, showing that the C terminus is required for TMEM18 to bind to DNA. This supports the model where the C terminus projects to the nucleus rather than into the cytoplasm [51]. The TMEM18 DNA-binding domain is only one α helix, and so the DNA target sequence for binding is expected to be quite short. TMEM18 binds to different 3 nucleotide words with different affinity (fig. 6), but there was no clear preference among 4 nucleotide words [51]. GCT and CTG were the most preferred binding sequences. Bound TMEM18 represses DNA transcription, possibly by simply physically excluding transcription factors from promoter regions. Clearly, binding to nucleotide trimers would be very non-specific, but the molecule readily dimerises providing a mechanism to increase specificity, whereby adjacent C-terminal ends of the protein would bind to repeated GCT elements, increasing the length of the target sequence

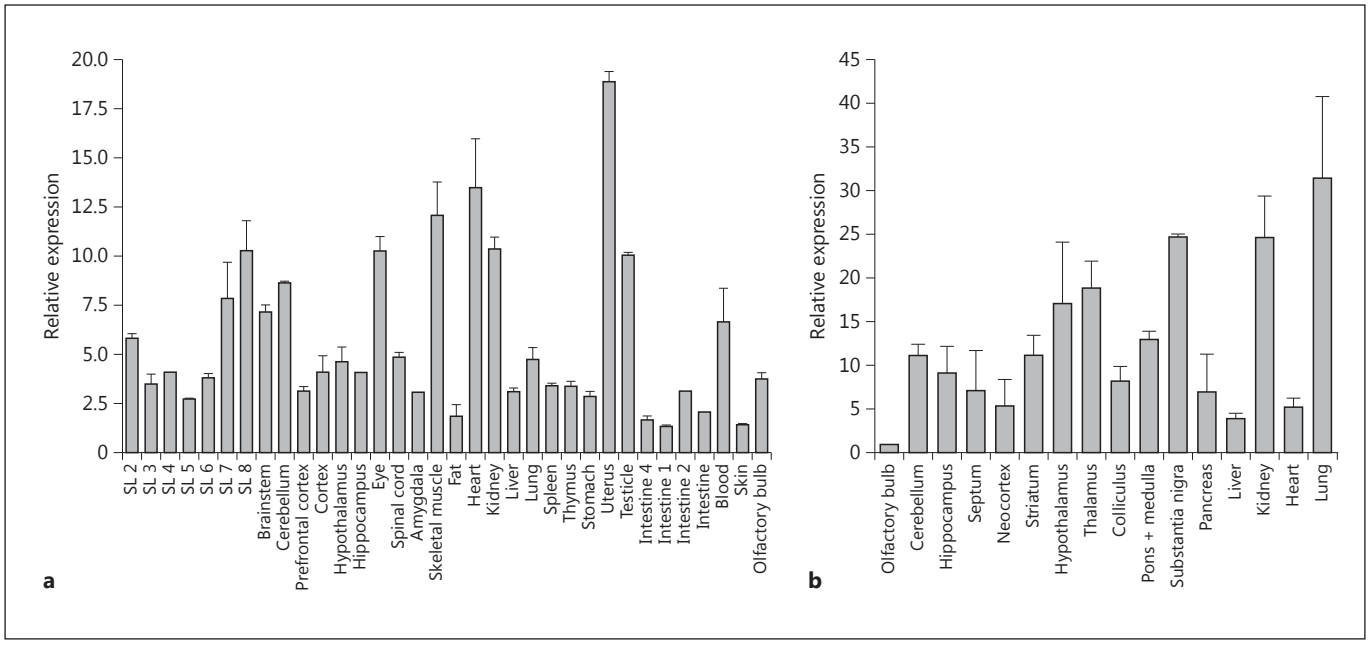

Fig. 5. Tissue-specific gene expression profiles for the gene TMEM18 in the rat (**a**) and mouse (**b**), relative to the lowest expressed tissue. The gene is expressed widely both within and outside the brain. See also figure 1 for tissue distribution of gene expression in humans [from 3]. SL 2–8 designate sequential coronal sections of the rat brain.

[51]. To date, however, specific DNA targets have not been identified, apart from the suggestion that it may suppress transcription in the transcriptional regulator Yin-Yang1 [51].

It therefore appears that TMEM18 sits on the nuclear membrane with a DNA-binding domain extending into the nucleus, which binds to specific binding sites, thereby regulating transcription. Given this model, an obvious potential mechanism by which TMEM18 may be linked to energy balance is via transcriptional repression of genes linked to food intake or energy expenditure. This viewpoint, however, is not supported by observations that there were no changes in the gene expression of TMEM18 in either the hypothalamus or brainstem of mice in relation to food deprivation (16 or 24 h), or to short (48 h) or long-term (3 weeks) exposure to a palatable dietary supplement: sucrose water or intralipid [3]. This lack of change in reported gene expression might be because TMEM18 is so widely expressed that TMEM18 levels in subpopulations of neurons are significantly altered, yet overall expression remains unchanged [3]. Another possibility, however, given the conserved glycosylation and phosphorylation sites, is that TMEM18 is mostly regulated posttranslationally rather than transcriptionally. Contrasting with the data for mice, it was observed that TMEM18 was downregulated in the hypothalamus of Long-Evans rats when fed a high-fat diet for 6 weeks [35]. This could be a species- or a diet-related difference, although as with the mice, starvation for 24 h did not affect expression levels of TMEM18 in the hypothalamus of the rats.

A further possibility is that given the distribution of TMEM18 among peripheral tissues, the relation of this gene to obesity is not via any centrally mediated action. In this sense, TMEM18 may be similar to Kilon/NEGR1, where the initial emphasis was on its central role, but has turned more recently to its potential role at the hub of a network in adipose tissue related to adipogenesis (discussed above). Supporting this alternative view, gene expression of TMEM18 in mice was reduced by 80% in adipose tissue during high-fat feeding [145], but brain levels were unaffected, as was observed by Almèn et al. [3]. TMEM18 expression was approximately doubled during adipocyte differentiation, in human adipocyte culture, and knockdown of the gene by siRNA approximately halved the rate of adipogenesis [13]. Insulin, IGF-1 and dexamethasone all significantly reduced gene expression of TMEM18 in adipose tissue, but there was no significant effect of isoprenaline [13]. In the periphery, in response to 6 weeks' feeding on a high-fat diet, TMEM18 expres-

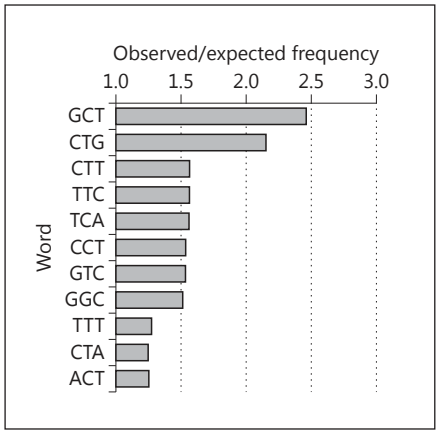

Fig. 6. Binding affinity of TMEM18 for different nucleotide triplets (words) on double-stranded DNA [from 51]. TMEM18 shows differential affinity for different 3-letter words but shows no differential affinity for 4-letter words. The differential affinity for DNA-binding sites may provide a mechanism by which TMEM18 acts as a transcriptional inhibitor.

sion was downregulated in the liver and soleus muscle of rats, but adipose tissue levels were unaffected [35].

Unsurprisingly, given its widespread distribution, TMEM18 has been implicated in several other disease states aside from obesity, notably in relation to cancer. Indeed, the gene was first identified as a terminal oligopyrimidine tract gene [142]. Terminal oligopyrimidine tract is an mRNA *cis*-regulatory sequence that inhibits translation from mRNA in growth-arrested cells. TMEM18 was also identified as a protein that enhances neural stem cell migration towards gliomas [52]. This action may be correlated with the suppression by TMEM18 of Yin-Yang1, which inhibits expression of genes that were increased in association with the attraction of neural stem cells to gliomas [51]. TMEM18 was also correlated with the tumorigenicity of human tumour-derived cell lines [1]. In addition to these links with cancer biology, *TMEM18* has been associated with the age of menarche [90], but the common polymorphisms near to *TMEM18* that have been linked to obesity were not associated with either education level or income [44].

In summary, *TMEM18* seems likely to play a role in obesity by transcriptional regulation of targets linked either to appetite and energy balance via its central sites of action or through an impact on adipogenesis in the periphery. The largest gaps in our knowledge relate to which genes TMEM18 may be transcriptionally regulating. Construction of transgenic or KO mice, combined with next-generation sequencing to identify transcriptomic profiles of the genetically manipulated versus wild-type mice, would be a large step forwards to enhance our understanding of its roles. A diagram summarising the potential role of *TMEM18* in obesity is shown in figure 7.

SNP	**rs9816226**
Chromosome	3q28
BMI link identified in	[116]
Location	Intronic
At risk allele	T
Nearest gene	ETS variant gene 5 (ETV5)
Synonyms	ERM
Entrez gene reference	2119
Ensembl gene reference	ENSG00000244405

The ETS gene family, comprising PEA3 and ER81, was first identified in the early 1990s in mice. These transcription factors share 95% identity in an 85 amino acid sequence, termed the ETS domain, which is responsible for DNA binding. In 1994, a new member of the family was discovered in humans and was called the ETS-related molecule (ERM) [78]. Gel shift analysis indicated that the full-length ERM protein was able to bind specifically to an oligonucleotide containing the consensus nucleotide core sequence GGAA that is recognised by the other ETS proteins. The gene was found to be almost ubiquitously expressed in normal human tissues, although it was particularly highly expressed in the brain and placenta, and to a lesser degree in the lung, pancreas and heart. In mouse tissues, it was also almost ubiquitously expressed [78]. ERM was subsequently renamed ETV5, although use of both names continues in the literature.

ERM/ETV5 is an oncogene with clear associations to several forms of cancer [reviewed in 85], notably prostate cancer, where chromosomal rearrangements of *ERM/ETV5* (and *ETV1* and *ETV4*) are thought to be one of the main factors in the genesis of prostate tumours. However, it is also implicated in Ewing's sarcoma, breast cancer and melanomas, and seems to be an important factor influencing the progression of endometrial cancer. *ERM/ETV5* has been shown to play a role in the normal development of the lungs [60], the kidney [61] and the limb buds [64]. It also appears to play a key role in normal spermatogenesis [49]. It is expressed in the granulosa and cumulus cells of mouse ovaries, during folliculogenesis, which it controls by regulating cyclooxygenase activity [26]. A promising link between ERM/ETV5 and genes linked to dopamine (DA) signalling was inferred from studies of *C. elegans*, in which it was shown that the ETS

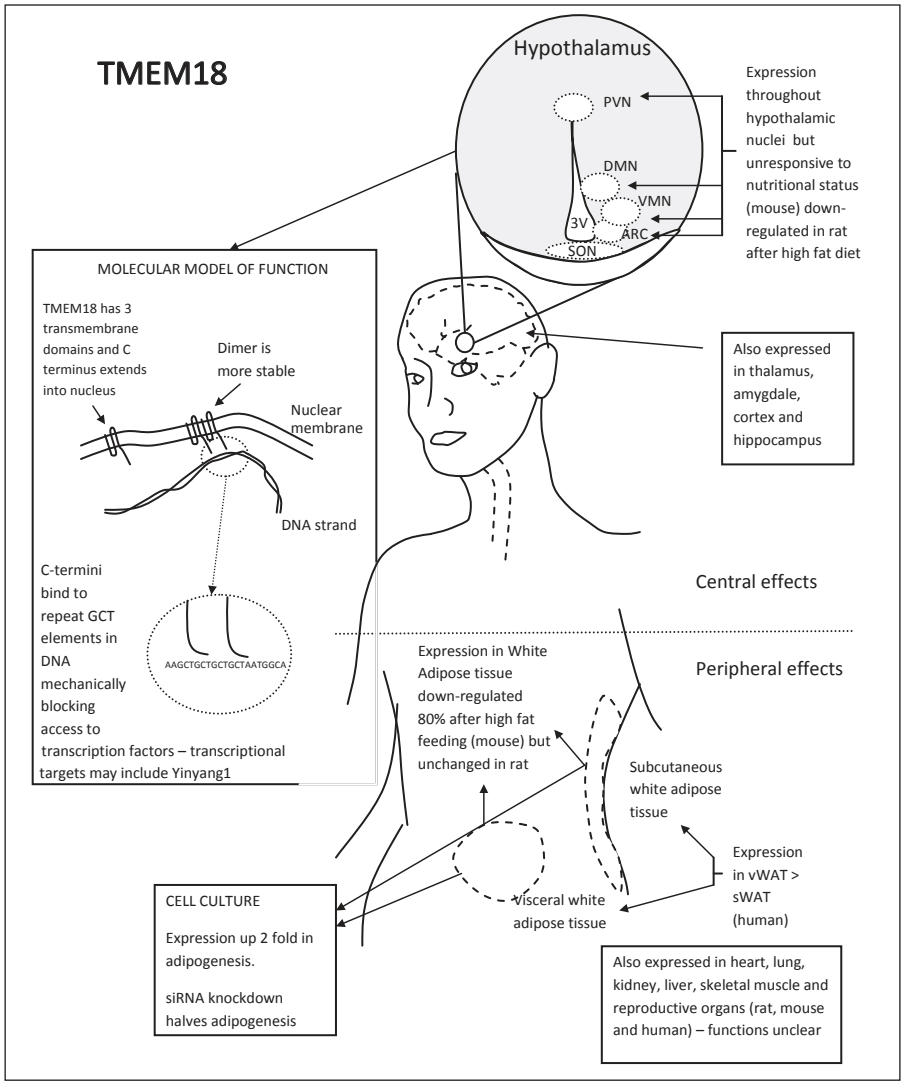

Fig. 7. Diagram summarising the effects of TMEM18 in relation to obesity.

family transcription factor ast-1 regulates multiple genes comprising the DA neuron phenotype, including biosynthetic enzymes and transporters [30]. DA signalling in the brain has been linked to obesity [131]; hence, if ERM/ETV5 was involved in the regulation of development of the DA system or regulation of its activity in the adult, this would provide a putative mechanism by which the link to obesity is mediated. This potential role for ERM/ETV5 is supported by the fact that it is highly expressed in the mouse midbrain [133]. However, it was shown in mice that ERM/ETV5 expression is not detectable until postnatal stages in the midbrain, well after development of the DA system [133]. Moreover, *ERM/ETV5* KO and control mice show comparable tyrosine hydroxylase and DA transporter expression in the embryonic and adult midbrain [133]. A link to obesity, via an impact on DA signalling, therefore seems unlikely. However, contrasting this view, nutritional state affected the gene expression levels of ERM/ETV5 in the substantia nigra/VTA in mice [15]. In the hypothalamus, ERM/ETV5 was downregulated in Long-Evans rats following 6 weeks of high-fat feeding, but it was unaffected by 24 h of starvation [35]. Expression levels in adipose tissue, liver, soleus and extensor digitorum longus (EDL) muscle were unaffected by high-fat feeding [35]. Similarly, nutritional state influenced gene expression of ERM/ETV5 in the ventromedial and arcuate nuclei of the hypothalamus in mice [15].

Being a transcription factor, the possibility that ERM/ETV5 influences energy balance by transcriptional regulation of key genes linked to food intake, in either the hypo-

thalamus or the substantia nigra/VTA, seems highly likely. For example, the GGAA-binding sequence for ERM/ETV5 is found in the NPY gene. However, we have no direct evidence for a link between the GWAS-identified SNPs and food intake or energy expenditure in humans. Moreover, despite the fact that mice that have had the *ERM/ETV5* gene knocked out have been in existence for at least 3 years [132], their metabolic phenotype with respect to obesity status remains unpublished. Given its wide tissue expression profile, the possibility that ERM/ETV5 also plays a direct role in the periphery cannot be discounted, especially as ERM/ETV5 seems to play a role in the regulation of serum concentrations of adiponectin [17].

SNP	**rs2112347**
Chromosome	5q13.3
BMI link identified in	[116]
Location	Intergenic
At risk allele	T
Nearest gene	FLJ35779
Synonyms	Centrosomal protein POC5
Entrez gene reference	134359
Ensembl gene reference	ENSG00000152359

POC5 is a 575 amino acid protein which appears to play an important role in centriole assembly [10]. Recruitment of POC5 occurs during the G2/M phase and continues up to full centriole maturation during the next cell cycle. Without POC5, RPE1 cells arrest in G1 phase, while HeLa cells show an extended S phase followed by cell death. POC5 does not appear to be needed for initiation of procentriole assembly, but is required for construction of the distal half of centrioles [10]. Apart from the linkage to obesity in GWAS studies, I could find no published literature pertaining to the function of this gene that might link it to obesity risk.

SNP	**rs10968576**
Chromosome	9p21.2
BMI link identified in	[116]
Location	Intronic
At risk allele	G
Nearest gene	Leucine-rich repeat and Ig domain-containing NOGO receptor-interacting protein (LINGO2)
Synonyms	LERN3, LRRN6C
Entrez gene reference	158038
Ensembl gene reference	ENSG00000174432

The *LINGO* family of genes (also called LERN and LRRIG family members) consists of 4 genes, all of which encode transmembrane proteins containing 12 extracellular leucine-rich repeats, an immunoglobulin C2 domain and a short intracellular tail. The family member *LINGO2/LERN3* that is linked to obesity in GWAS is expressed exclusively in the brain [46]. During embryogenesis in the mouse, LINGO2/LERN3 is expressed in a population of cells lying adjacent to the epithelial lining of the olfactory pit [36]. In the adult mouse brain, it is expressed exclusively in neuronal tissue. The LINGO proteins are known to interact with the NOGO receptor [73], which is involved in the regulation (primarily inhibition) of neurite outgrowth. Inhibition of LINGO1 increases the survival of dopaminergic neurons in models of Parkinson's disease [48]. Variants of *LINGO2* have been linked to essential tremor in Parkinson's disease [126, 128]. Hence, a linkage to obesity via interactions with DA signalling seems a possibility. Otherwise, we have very little information about the function of this gene.

SNP	**rs7498665**
Other significant nearby SNPs	rs147094247, rs8049439, rs4788102, rs7498665, rs60604881, rs62037368, rs62037369
Chromosome	16p11.2
BMI link identified in	[19, 116, 137]
Location	Exonic
At risk allele	G
Nearest gene	Src-homology 2B adaptor protein 1 (SH2B1)
Synonyms	PSM SHB-2 KIAA1299
Entrez gene reference	25970
Ensembl gene reference	ENSG00000178188

The SH2B adaptor protein 1 *(SH2B1)* is unusual among the genes linked to obesity via GWAS, because the main identified SNP is exonic. The SNP rs7498665 is non-synonymous and leads to an alanine to threonine amino acid substitution at residue 484 of the 767 AA protein. SH2B1 can be spliced in four different ways, generating α, β, γ and δ forms [96]. It is also unusual among the genes reviewed here in that a clear functional link of the gene to signalling systems related to energy balance and obesity was well established before the gene was identified as being associated to BMI and adiposity by the GWAS. *SH2B1* is expressed in numerous mouse tissues, including the brain, hypothalamus, liver, muscle, adipose tissue, heart and pancreas [54, 96], with different splice variants being differently represented in the different tissues [96].

In humans, expression was highest in the skeletal muscle, pituitary gland, lymphocytes, kidneys, ovaries and pancreas (fig. 1) [13]. Expression in the brain and hypothalamus was below the median expression across all tissues, which is surprising in the light of previous studies relating to its function as a modulator of the JAK-STAT signalling pathway.

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway is a major intracellular signalling pathway that links cell surface receptors to transcriptional targets in the nucleus. This includes multiple hormones and cytokines, including the receptors for growth hormone (GH), leptin, insulin and erythropoietin. JAK is a tyrosine kinase that can bind to the intracellular domain of cytokine and other receptors. When the receptor binds to its ligand, the JAK protein is activated, resulting in phosphorylation of tyrosine residues on the receptor by JAK. Proteins that contain phosphotyrosine Src homology 2 (SH2) domains can bind to these sites. One class of proteins containing such sites are the STAT proteins. Once bound to the receptor, the STAT proteins can themselves be phosphorylated by JAK at tyrosine residues, allowing other STAT proteins to recruit onto them, and then dimerise and release from the receptor. These dimerised STAT proteins can then translocate to the nucleus, where they initiate transcription. There are 5 STAT proteins, and hence a variety of alternate dimer products, that can initiate transcription at a range of different transcriptional targets.

The JAK-STAT pathway is negatively regulated by two sets of molecules. The suppressors of cytokine signalling (SOCS) are transcriptionally activated by STAT proteins, and once translated, the mature SOCS proteins compete with STAT for phosphotyrosine binding sites or reduce signalling by inhibiting JAK protein activity. Alternatively, protein tyrosine phosphatases (PTPs) can inhibit signalling by dephosphorylating the sites phosphorylated by JAK. In contrast, the pathway activity can be enhanced by another family of molecules, which themselves contain SH2 homology domains, called the SH2 adaptor proteins. These include *APS* (later renamed *SH2B2*), *Lnk* (renamed *SH2B3*) and *SH2-B* or *PSM* [99] (later renamed *SH2B1*). SH2B1 binds to unphosphorylated JAK at the leptin receptor (in the absence of leptin), via its non-SH2 domain regions. The mechanism by which SH2B1 enhances the activity of JAK is by performing a common adaptor protein role, whereby it helps to recruit target proteins to the receptor (e.g. STATs). However, SH2B1 also appears able to directly enhance the kinase activity of JAK proteins by up to 20-fold [66, 105].

JAK2 has a total of 49 tyrosines in the mouse [103], which, if auto-phosphorylated, could also serve as docking sites for SH2 domain-containing molecules. When leptin binds to its receptor, it stimulates phosphorylation of tyrosine residue 813 on JAK2, which subsequently binds to the SH2 domain of SH2B1 [58]. This binding of SH2B1 to JAK2, at tyrosine 813, enhances the leptin induction of JAK2 activity. It has been suggested that phosphorylation of tyrosines 868, 966 and 972 in the kinase domain of JAK2 is required for JAK2 to retain maximal kinase activity [8]. This is based on the observation that JAK2, lacking tyrosines at either 868, 966 or 972, has substantially reduced activity. However, co-expression with SH2B1β partially restores the activity of all three JAK2 mutants. Based on these results and the crystal structure of the JAK2 kinase domain, Argetsinger et al. [8] suggested that small conformation changes of JAK2, adjacent to tyrosines 868, 966 and 972, may be essential for JAK2 to assume a maximally active conformation. These small conformation changes may include phosphorylation, binding to a ligand-bound cytokine receptor and binding to SH2B1. This ability of SH2B1 to directly enhance the kinase activity of JAK appears to be unique among adaptor proteins [66].

The long form of the leptin receptor (LepR-b) is a type 2 cytokine receptor with an intracellular domain that is linked to a JAK2-STAT3 signalling cascade. Given that SH2B1 is a positive regulator of this signalling pathway, it is not surprising that mice with the SH2B1 gene knocked out had impaired leptin signalling, resulting in obesity, hyperlipidaemia and leptin resistance [79, 95, 96] (fig. 8). Food intake was increased by about 60% in the KO animals [95]. In addition to this increase in food consumption, there was also an unexpected increase of oxygen consumption per gram of tissue in the KO compared to the wild-type animals [95]. Ren et al. [95] suggested that, despite the elevated energy expenditure, the KO mice were still in a state of greater positive energy balance than control wild types, explaining their greater obesity. However, the calculations of the energy imbalance of the KO animals (15 kcal per day) are at odds with the rise in body weight. If the mice really were consuming 15 kcal per day more than they expended, then one would expect them to increase in mass by over 1 g per day, yet over the 20 weeks (140 days) from age 7 to 27 weeks, the mice increased by only 40 g. Even assuming the calculated intake did not account for faecal and urinary losses does not account for the difference.

The data on food intake and energy expenditure in Ren et al. [96] are even less clear. In this case, food intake

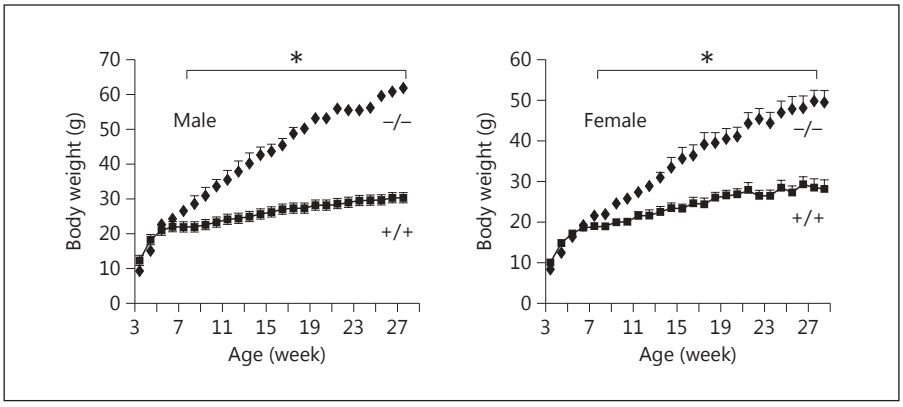

Fig. 8. Body weight change of male and female wild-type (+/+) mice and mice with whole-body KO of the SH2B1 gene (–/–). Knocking out the gene leads to the development of severe obesity in both sexes [from 95].

of the KO animals increased by about 30%, but their energy expenditure (per gram) almost doubled. The estimated energy expenditure for the two groups derived from data in the figures shows that the wild-type mice were in positive energy balance (intake about 20.8 kcal/day compared to an expenditure of about 14.5 kcal/day), but the KO animals were not (intake of about 26.5 kcal/day and expenditure of about 35.2 kcal/day). The data collected by Morris et al. [79] have a similar anomaly (for wild type, intake of about 18 kcal/day against an expenditure of about 13 kcal/day and in the KO mice, an intake of about 26 kcal/day against an expenditure of about 31 kcal/day). Clearly, more data are required to establish exactly how an energy imbalance leading to obesity develops in these mice.

The impairment in the KO mice resulting in obesity is exclusively a consequence of the loss of SH2B1 in the brain [79, 96]. Ren et al. [96] created transgenic mice expressing rat SH2B1β in the brain and crossed these with a whole-body KO mouse to generate mice expressing SH2B1 only in neural tissue. Neuron-specific restoration of SH2B1β not only corrected the metabolic disorders in constitutive KO mice, but also improved JAK2-mediated leptin signalling and leptin regulation of orexigenic neuropeptide gene expression in the hypothalamus. Moreover, neuron-specific overexpression of SH2B1 protected against high-fat diet-induced leptin resistance and obesity, via a normalisation of both food intake and energy expenditure (oxygen consumption) to levels observed in wild-type animals fed chow diet. Morris et al. [79] illustrated the same phenomenon by generating three different lines of mice, expressing wild-type, SH2 domain-defective (R555E) or SH2 domain-alone (Delta N503) forms of SH2B1, specifically in neurons. These mice were then crossed with SH2B1 KO mice to generate KO/SH2B1, KO/R555E, or KO/Delta N503 compound mutant mice. In these mice, there was no peripheral SH2B1, but various forms of neuronal SH2B1. Neuron-specific expression of wild-type SH2B1, but not R555E or Delta N503, corrected hyperphagia, obesity, glucose intolerance and insulin resistance in SH2B1 null mice. These data showed that the effects of knocking out SH2B1 globally were entirely due to its central actions. Moreover, since the Delta N503 construct did not rescue the obesity phenotype, these data indicated that, in addition to the SH2 domain, N-terminal regions of neuronal SH2B1 are also required for the maintenance of normal body weight and glucose metabolism.

In addition to the common polymorphisms identified by GWAS, a number of rare loss-of-function mutations have also been identified in the *SH2B1* gene in humans [23, 127] as well as in a number of patients with large deletions of sections of chromosome 16 that encompass the gene [14, 130]. All cases, carriers of the loss-of-function mutations as well as cases with gene absence, exhibited hyperphagia, childhood onset obesity, disproportionate insulin resistance and reduced final height as adults. The effect on energy intake was consistent with data from 1,700 Dutch females where the 'at risk' SNP in SH2B1 was linked with increased fat consumption [12].

Given this background, the role of impairments of this gene in causing obesity would appear to be clear. Impaired SH2B1 removes the enhancement of intracellular leptin signalling in the brain, leading to a blunting of the downstream response to leptin (leptin resistance), which generates hyperphagia and hence obesity. However, that said, the role of the specific exonic SNP mutation identified by GWAS, and the other GWAS SNPs that are intronic or intergenic, is far less clear. First, the exonic SNP-driven protein modification does not localise to the SH2 domain, where an impact on binding to

JAK might be anticipated. Given the finding that other parts of the molecule may also be linked to its function as an enhancer molecule [79, 96], this is not completely surprising. Nevertheless, the JAK2-STAT3-mediated leptin signalling of HEK293 cells carrying the Ala464Thr SNP in vitro was not impaired compared to the wild-type control [127]! Rather surprisingly, it was also found that nutritional state had no effect on the gene expression levels of SH2B1 in the ventromedial and arcuate nuclei of the hypothalamus (and the substantia nigra/VTA) in the mouse [15]. This suggests there may be a much more subtle impact of the gene on energy balance, the details of which we are currently unaware. These could conceivably include actions in the periphery or on brain development.

In the periphery, *SH2B1* plays a major role in insulin signalling, as might be expected given the fact that JAK-STAT signalling is also an intracellular signalling pathway used by the insulin receptor. SH2B1 (α) is highly expressed in the pancreas as well as the liver, skeletal muscle and adipose tissue, which are the major sites of insulin action. Early work suggested that SH2B1 does not bind directly to phosphorylated tyrosine residues on the insulin receptor substrate (IRS) proteins [54]. Instead, it was suggested that the protein interacted with the activation loop of the receptor [54]. This suggestion was supported by work showing that a panel of single-point mutants of the activation loop, specifically Tyr(1158), Tyr(1162) and Tyr(1163), all abolished interaction with SH2B1. It has also been suggested that the primary association between SH2B1 is at the C terminus [99, 132]. Nevertheless, later studies suggested that the protein does bind directly to IRS-1 and IRS-2, as well as to the insulin receptor itself [80], and the binding to IRS-1 and IRS-2 was stimulated by insulin. In mice, where the peripheral SH2B1 was knocked out but the brain expression was intact, there were profound negative effects on insulin signalling across multiple tissues [80]. The knock-on effect of this impairment was that deletion of peripheral SH2B1 markedly exacerbated high-fat diet-induced hyperglycaemia, hyperinsulinemia and glucose intolerance.

In vitro, the deletion of SH2B1 impaired insulin signalling in primary hepatocytes, whereas SH2B1 overexpression stimulated insulin receptor auto-phosphorylation and tyrosine phosphorylation of IRSs. The SH2 domain of SH2B1 was required, and sufficient, to promote insulin receptor activation [22]. The physical interaction between SH2B1 and the IRS proteins inhibited tyrosine dephosphorylation of IRS-1 or IRS-2 and thereby increased the ability of the IRS proteins to activate the phosphatidylinositol 3-kinase (PI3K) pathway. The polymorphism rs7359397, identified by GWAS, has been linked to variation in the HOMA index of insulin sensitivity [28].

SH2B1 also seems to play an important role as a modulator of nerve growth via an interaction with nerve growth factor (NGF) receptor [94]. NGF is essential for the development and survival of sympathetic and sensory neurons. NGF binds to the TrkA receptor, activates the intrinsic kinase activity of TrkA and promotes the differentiation of pheochromocytoma (PC12) cells into sympathetic-like neurons. In culture, overexpression of SH2B1 with the SH2 domain mutated blocked NGF-induced neurite outgrowth of PC12 cells. In contrast, overexpression of wild-type SH2B1 enhanced NGF-induced neurite outgrowth [104]. Interestingly, the overexpression of either wild type or the mutated SH2B1 did not alter tyrosine phosphorylation of TrkA, Shc or phospholipase Cγ in response to NGF, or modulate NGF-induced activation of ERK1/2. This suggested that SH2B1 was not interacting with the NGF receptor in the same way that it interacts with cytokine receptors, like the leptin receptor (detailed above). Supporting the suggestion that SH2B1 acts with a different mechanism with respect to NGF, depleting endogenous SH2B1 using short hairpin RNA inhibited NGF-dependent neurite outgrowth in PC12 cells, but had no effect on NGF-mediated phosphorylation of the downstream intracellular signals Akt or ERKs 1/2 [67]. In fact, SH2B1 has a nuclear localisation signal in its sequence [67]. Mutation of this signal had no effect on NGF-induced activation of TrkA and ERKs 1/2, but prevented SH2B1β from enhancing NGF-induced neurite outgrowth. It thus appeared that SH2B1 was acting as a transcription factor, shuttling into the nucleus to directly activate transcription [67]. Supporting this view, disruption of the SH2B1β nuclear import signal also prevented SH2B1β from enhancing NGF-induced transcription of genes, which are important for neuronal differentiation [67]. Phosphorylation of serine residues in the vicinity of the nuclear localisation sequence is important in regulating its cellular location [68].

SH2B1 enhances neurite outgrowth in at least three other ways. First, it acts as an adapter protein for FGF-mediated neurite outgrowth and glial cell line-derived neurotrophic factor (GDNF)-induced neurite outgrowth by prolonging both FGF and GDNF signalling [59]. In addition, SH2B1 plays a role in the regulation of N-cadherin, a molecule known to be involved in the engagement between cells that contributes to neuronal migra-

tion. Overexpression of SH2B1β reduced N-cadherin levels and increased phosphotyrosine 654 β-catenin, leading to increased NGF-induced neurite initiation in PC12 cells [134]. In contrast, overexpression of the mutant SH2B1 lacking the SH2 domain increased N-cadherin expression, cell-cell aggregation, and reduced neurite initiation. SH2B1 seemed to do this by binding directly to the N-cadherin [134].

Given its critical role as an enhancer protein on a wide variety of receptors, it is unsurprising that SH2B1 has been implicated in several other physiological and disease processes. These include the regulation of bone mineral density [141], cancer [146], erythropoiesis [50], prolactin signalling [98] and hence control of the actin cytoskeleton [97]. Unexpectedly, carriers of the loss-of-function mutations linked to obesity also exhibited a spectrum of behavioural abnormalities that were not reported in controls, including social isolation and aggression [23].

In summary, there is considerable evidence showing that SH2B1 acts as an adaptor protein that enhances signalling in the JAK-STAT pathway, downstream of the leptin receptor. In the hypothalamus, this action enhances leptin signalling and protects against obesity development. Knocking out the gene in animals or natural loss-of-function mutations in humans both lead to elevated food intake and severe obesity associated with leptin resistance. The impact of the KO on energy expenditure is unclear. Moreover, the functions of the specific polymorphisms identified by GWAS remain less certain. In cell culture, the 'at risk' SNP did not appear to affect leptin signalling via the JAK-STAT pathway, as might have been anticipated. The gene also plays a role as an enhancer of insulin signalling, downstream of the insulin receptor, and in neurite outgrowth, due to multiple actions including a role as a transcription factor, downstream of the nerve growth receptor (NGR). Hence, it could play a role in the development of the neural circuitry controlling feeding behaviour. Carriers of the 'at risk' SNP appear to have increased food consumption. However, it would not be surprising, given its role in mediating the responses of both leptin and insulin receptors, if it was shown to also play a significant role in adipogenesis. The gene was neither up- nor downregulated during adipogenesis [13]. This does not necessarily suggest that it has no role, since its hypothalamic expression status was also unresponsive to nutritional state [15] and it undoubtedly plays an important part in the control of food intake. A diagram summarising the functions of SH2B1 in relation to obesity is shown in figure 9.

SNP	**rs2287019**
Chromosome	19q13.32
BMI link identified in	[76, 102]
Location	Intronic
At risk allele	C
Nearest gene	Gastric inhibitory polypeptide receptor (GIPR)
Synonyms	Glucose-dependent insulinotropic polypeptide receptor, gut-derived nutrient intake polypeptide receptor
Entrez gene reference	2696
Ensembl gene reference	ENSG00000010310

Discovered in 1971 [18], GIP was originally called the gastric inhibitory polypeptide, but it is more recently referred to as the glucose-dependent insulinotropic polypeptide. It is a 40 AA incretin hormone secreted by the duodenal endocrine K cells [93]. Like the other main incretin hormone, GLP-1, it mediates enhanced release of insulin from the pancreas [24] in response to high levels of glucose (or fat) in the duodenum. The receptor for this hormone (GIPR) is a G-protein coupled receptor, primarily localised to β cells in the pancreatic islets, where it exerts its insulinotropic action. Knocking out the GIPR gene in mice resulted in greater levels of circulating glucose and an impaired immediate response to an oral glucose load, which is consistent with its role as an incretin [77]. However, the overall impact on glucose tolerance was relatively modest, due in part to compensatory increases in the activity of the GLP1 signalling pathway, related to altered islet structure [88]. Surprisingly, knocking out both *GIPR* and *GLP-1R* in the same mouse did not produce a more profound glucose intolerance, indicating other compensatory measures are also enabled [38]. In all these cases, the genetically manipulated mice had a normal body weight phenotype.

Extrapancreatic populations of the receptor are also found in the brain, on bone and on adipocytes [139], and, as might be expected, in these other locations, it exerts rather different effects. Miyawaki et al. [76] showed that GIPR KO mice fed on a high-fat diet were resistant to weight gain. Moreover, when the mice were cross-bred with ob/ob mice lacking functional leptin, which normally become profoundly obese, the extent of weight and adiposity gain was blunted. In part, this was because the GIPR KO mice used fat as a preferred energy substrate (reflected in a lowered respiratory quotient). Similar results were found in the double KO animals with loss of both *GIPR* and *GLP-1R* [38]. In addition to the switch towards greater fat oxidation in these double KO mice, they also exhibited increased energy expenditure linked

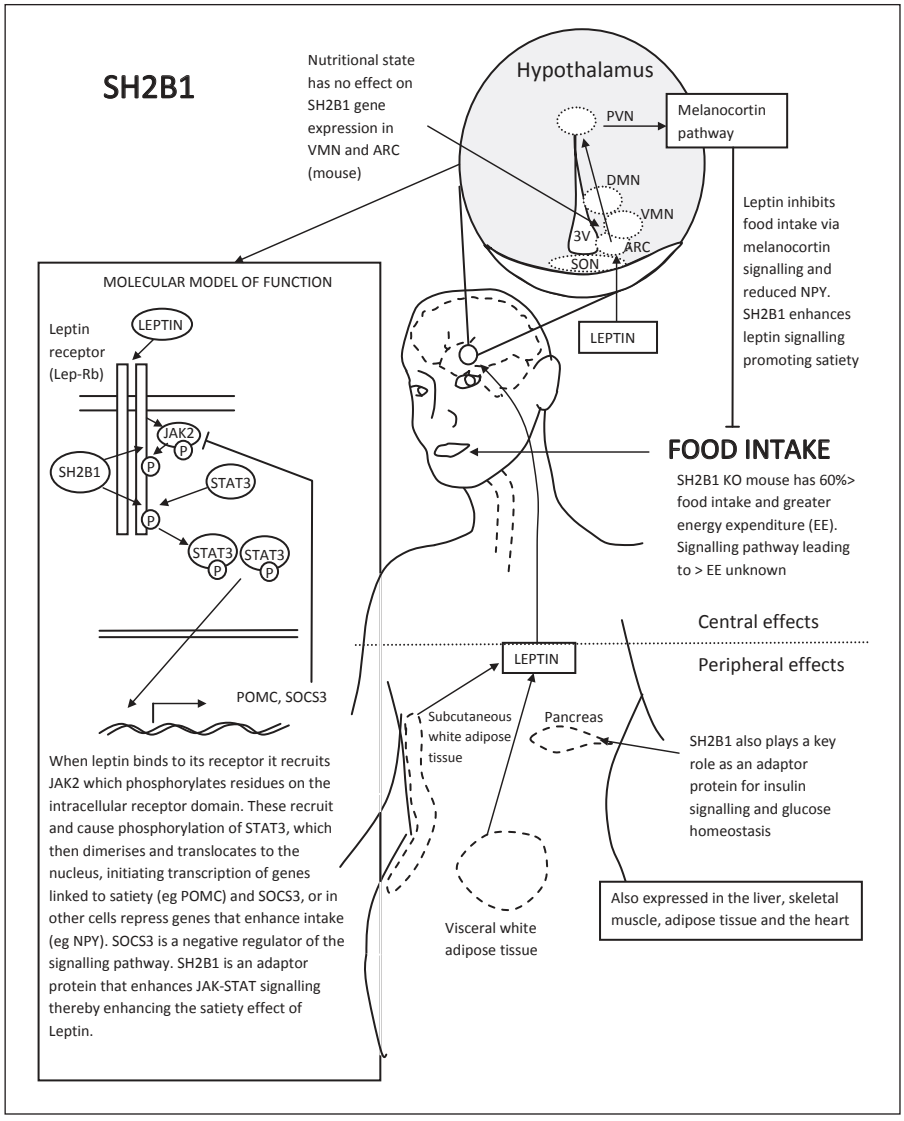

Fig. 9. Diagram summarising the effects of SH2B1 in relation to obesity.

to greater physical activity levels [39]. Yamada et al. [140] showed that this elevated physical activity in GIPR KO mice was maintained throughout life and protected these mice from the age-associated increase in body fatness and its consequences for age-related insulin resistance. Consistent with the maintained lower body fatness, the levels of adiponectin were greater in the GIPR KO mice [140]. This elevation of adiponectin levels in GIPR KO mice has been suggested to be instrumental in driving both the increase in energy expenditure and fat oxidation in skeletal muscle [81]. In addition to these genetic manipulations, several other papers have pointed to an effect of GIPR in the regulation of body weight and fatness. For example, administration of GIPR peptide antagonists [69], immunisation against GIP [32] or ablation of the intestinal K cell where GIP is produced [4] all result in protection against high-fat feeding-induced weight gain.

There are two main potential mechanisms through which intact GIPR may promote obesity in the face of feeding on a high-fat diet. GIPR on the adipocytes may act to enhance uptake and storage of circulating triglycerides, matched with a stimulation of adipogenesis. Several lines of evidence support such a direct role. In culture, GIP inhibits lipolysis [34, 43], acting via adipocyte-specific receptors [101, 144]. However, in vivo, the action may be indirect and mediated via the GIPR stimulation of insulin, which then promotes adipogenesis and fat uptake. Indeed, the stimulation of lipid uptake by GIP in

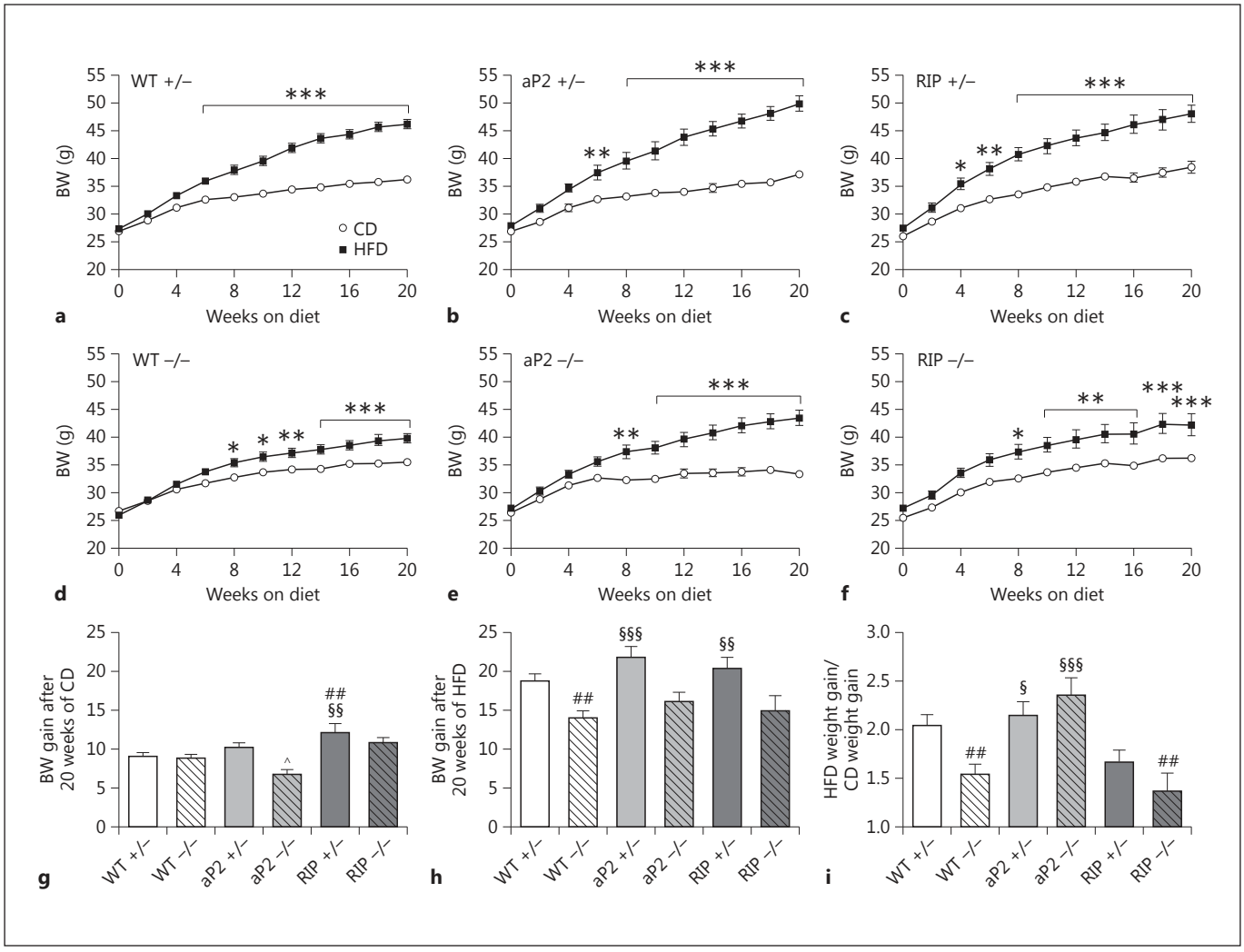

Fig. 10. Effects of expressing human GIPR in the adipose tissue (aP2+/–) or β cells (RIP+/–) of mice that had endogenous GIPR knocked out (WT–/–). The aP2 (–/–) and RIP (–/–) are mice null for human GIPR and mouse GIPR. Weight gain on a high-fat diet was reduced when the GIPR was knocked out (compare **a** and **d**). Repletion of the GIPR in either the adipocyte (**b**, **e**) or in the β cell (**c**, **f**) removed this protection from diet-induced obesity [from 123]. BW = Body weight; CD = control diet; HFD = high-fat diet. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, HFD versus CD; ## $p < 0.01$, versus WT (+/–) fed the same diet; § $p < 0.05$, §§ $p < 0.01$, §§§ $p < 0.001$, versus WT (–/–) fed the same diet; ^ $p < 0.05$, aP2 –/– versus aP2 +/–.

culture is enhanced when insulin is present [43], indicating both actions may play a role. To address this issue in vivo, mice were generated that had targeted tissue-specific expression of human GIPR in only the adipocytes (aP2+/–) or in only the β cells (RIP+/–) [123]. By crossing these mice with GIPR KO mice, they generated mice that expressed human GIPR in adipocytes or β cells only. It was suggested from these data that mice with active GIPR in adipocytes gained weight at the same rate as controls, when fed a high-fat diet, and at a significantly higher rate than the weight gain of the global KO animals. However, mice with expression of GIPR only in the β cells did not gain weight to the same extent. These interpretations, however, depend very critically on how the weight gain was expressed. The above interpretations were generated by dividing the weight gain when fed the high-fat diet by the weight gain when fed the control diet (fig. 10i) [123]. However, this approach generates an anomalous result, because the response to the high-fat diet in this case is greatest in the aP2–/– mice, which should have no active GIPR of either human or mouse origin and hence should not differ from the wild-type KO animals. This difference

seems to be an artefact of dividing by the slightly lower weight gain of the mice on the control diet. Equally, the low gain of the RIP (+/–) mice (expressing human GIPR on the β cells) seems only to be generated by the slightly higher rate of weight gain in the controls. If one examines just the weight gain after 20 weeks of high-fat diet feeding (fig. 10h), then it is clear that the weight gains of the mice without the human or mouse GIPR (i.e. WT–/–, aP2–/– and RIP–/–) were all similar (see also fig. 10d–f). In contrast, the weight gains of the mice either with intact native GIPR in all tissues (WT+/–), human GIPR in adipose tissue (aP2+/–) or human GIPR in β cells (RIP+/–) were also all similar (see also fig. 10a–c). This suggests that both direct and indirect modes of action for GIPR on fat storage play an important role.

Ugleholdt et al. [123] additionally suggested that the differences in weight gain on a high-fat diet were due to differences in lean rather than fat tissue (see original paper fig. 3), although this again relies on dividing the changes under high-fat feeding by those observed under the control diet, which produces similar anomalous high responses in the aP2–/– group. In contrast, using the raw data for the response to a high-fat diet alone indicates that the WT+/–, aP2+/– and RIP+/– groups have consistently gained 4–5 g more body fat under a high-fat diet when compared with the WT–/–, aP2–/– and RIP–/– animals, respectively, suggesting that at least 50% of the impact of a high-fat diet on total weight gain was contributed to by differences in fat mass. While impacts on lean mass clearly also contribute to the total weight change, these do not explain the entire impact on adiposity.

An impact of GIP on bone would be anticipated because it stimulates insulin production, which influences bone formation and mineralisation [125]. However, GIPR are also found on osteoblasts [16], suggesting that GIP may also have a direct role in bone metabolism. Indeed, treatment of cultured osteoblasts stimulates cellular events linked with bone formation. This might be significant since bone metabolism contributes to total energy metabolism and hence may influence energy balance. An impact of GIPR loss on bone metabolism in vivo was first shown in the GIPR KO mouse [138]. These mice had reduced bone size, lower bone mass, altered bone microarchitecture and biomechanical properties, and altered parameters for bone turnover, especially bone formation. In addition, the mice had earlier age-related changes than wild-type mice in body composition, including bone mass. Tsukiyama et al. [120] suggested that these effects occurred because GIP (acting on GIPR in bone) directly influences calcium uptake into bone, facilitating utilisation of dietary calcium in circulation, which is normally elevated after a meal.

These responses then suggest that GIP, in concert with GIPR, plays a general role in facilitating the utilisation of ingested nutrients. This includes increasing insulin to dispose of ingested glucose, increasing fat uptake into adipocytes and stimulating adipogenesis, and finally stimulating calcium uptake and bone formation in bony tissue. In fact, on this basis, it has been suggested that it would be better called gut-derived nutrient intake polypeptide [139]. In addition to these effects, GIP seems to have an impact on appetite via GIPR receptors in the brain. In humans, where insulin was clamped, intraduodenal glucose infusion led to a rise in GIP, followed by GLP-1 [55]. Decreases in appetite were closely linked to GLP-1 levels. Subsequent data indicated that because GLP-1 is rapidly degraded before it reaches the systemic circulation, this action is probably because GLP-1 stimulates sensory afferents in the gastrointestinal mucosa, which signal to both the brain stem and hypothalamus [45]. Nevertheless, direct infusions of physiologically relevant levels of GLP-1 were effective in reducing appetite [31], pointing to a dual mechanism. In contrast, GIP infusion in humans also impacted appetite sensations, but in the opposite direction [20]. This increase in appetite occurred despite the fact that it was later shown that GIP infusion in humans probably decreases production of ghrelin [102], another gut hormone that is produced by the stomach, and stimulates appetite. Unexpectedly, GIP infusion also caused a reduction in metabolic rate [20].

In the brain, GIPR is expressed in the hypothalamus, hippocampus and cortex [111]. GIPR KO mice have impaired learning abilities, particularly related to defects in spatial learning and memory as evaluated by the Morris water maze [27]. Infusion of GIP into the hypothalamus of rats for 4 days resulted in upregulation of hypothalamic mRNA levels of several genes related to food intake and energy balance, including AVP, CART, NPY, OXT and STAT3 [5]. In GIPR KO mice, gene expression of AVP, CART, OXT and STAT3 in the hypothalamus were downregulated relative to wild-type mice [5]. These data suggest that stimulation of AVP and NPY may underpin the stimulatory effect of infused GIP on appetite [20]. The positive impact on CART and OXY levels, however, is confusing, because CART and OXT are anorexigenic agents.

Like SH2B1, GIPR is unusual among the genes linked to BMI and adiposity by GWAS studies in that a clear link to obesity was established before the gene was identified by GWAS. The manner in which the intronic SNP influences the impact of GIPR remains uncertain. However, it

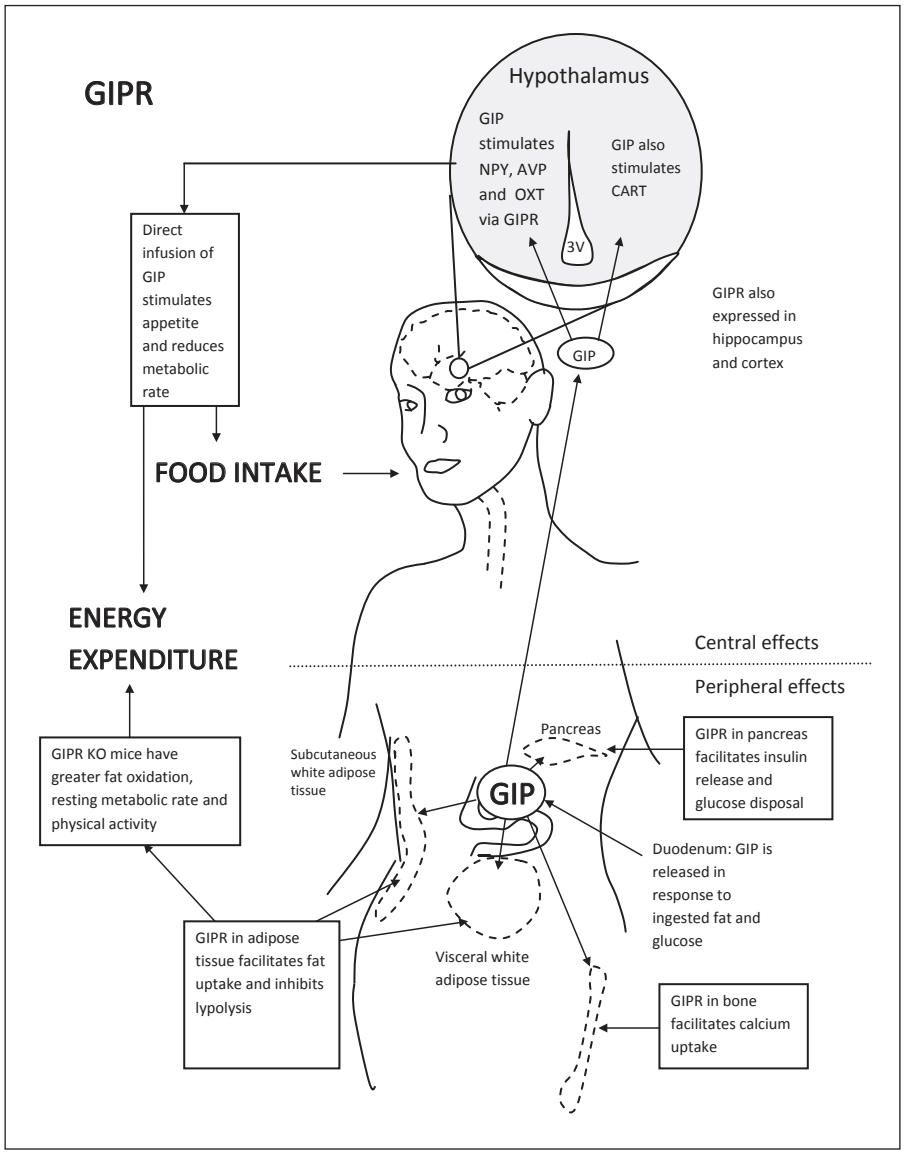

Fig. 11. Diagram summarising the effects of GIPR in relation to obesity.

seems likely that some impact on the ability of the receptor to stimulate adipogenesis and fat uptake, or on the actions of the GIP hormone in the brain on appetite, are probable. The effect on energy expenditure remains paradoxical and still requires a mechanistic explanation. A summary of the effects of GIPR in relation to obesity is offered in figure 11.

Conclusions

Of the 7 genes reviewed here, only 2 had clear associations with obesity before the advent of the GWAS studies. Even in these 2 genes, where we know something about how they influence energy balance and fat storage, the mechanism(s) by which the specific polymorphisms identified by GWAS act to cause weight gain remain unclear. For the other genes, where a linkage to obesity was not previously obvious, we are even less able to specify how the association comes about. All 7 genes are expressed in the brain. Two of these, *SH2B1* and *GIPR,* have been linked directly to appetite regulation. However, 3 of the genes *(NEGR1, SH2B1* and *LINGO2)* have also been implicated in neurite outgrowth. Therefore, rather than a direct contribution as part of the appetite signalling system, SNPs nearby these genes may primarily contribute to weight regulation and obesity by influencing the devel-

opment of the energy balance regulatory circuits in the brain – and hence the polymorphisms may effectively be silent in adulthood. In spite of this apparent domination of central effects [see also 87, 139], it has recently become clear that all of these targets, where we have any detailed information, have complex functions in multiple tissues. Notably, 4 of the genes *(Kilon/NEGR1, TMEM18, SH2B1* and *GIPR)* are also expressed in adipocytes. It could well be that the SNPs in the neighbourhood of these genes exert their actions via effects on the peripheral gene functions, rather than the central functions. Such action outside of the hypothalamus has also recently been inferred for the effect of FTO on body composition [70].

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Erratum

In the article 'Functional Analysis of Seven Genes Linked to Body Mass Index and Adiposity by Genome-Wide Association Studies: A Review' by Speakman (Hum Hered 2013;75:57–79) reference [123] to figure 10 was omitted.