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REVIEW

What model organisms and interactomics can reveal about the genetics of human obesity

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Abstract Genome-wide association studies have identified a number of genes associated with human body weight. While some of these genes are large fields within obesity research, such as *MC4R*, *POMC*, *FTO* and *BDNF*, the majority do not have a clearly defined functional role explaining why they may affect body weight. Here, we searched biological databases and discovered 33 additional genes associated with human obesity (*CADM2*, *GIPR*, *GPCR5B*, *LRP1B*, *NEGR1*, *NRXN3*, *SH2B1*, *FANCL*, *GNPDA2*, *HMGCR*, *MAP2K5*, *NUDT3*, *PRKD1*, *QPCTL*, *TNNI3K*, *MTCH2*, *DNAJC27*, *SLC39A8*, *MTIF3*, *RPL27A*, *SEC16B*, *ETV5*, *HMGA1*, *TFAP2B*, *TUB*, *ZNF608*, *FAIM2*, *KCTD15*, *LINGO2*, *POC5*, *PTBP2*, *TMEM18*, *TMEM160*). We find that the majority have orthologues in distant species, such as *D. melanogaster* and *C. elegans*, suggesting that they are important for the biology of most bilateral species. Intriguingly, signalling cascade genes and transcription factors are enriched among these obesity genes, and several of the genes show properties that could be useful for potential drug discovery. In this review, we demonstrate how information from several distant model species, interactomics and signalling pathway analysis represents an important way to better understand the functional diversity of the surprisingly high number of molecules that seem to be important for human obesity.

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Introduction

Tremendous progress has been made in the field of obesity research, with academic and industrial interest growing rapidly. During the 1980s (1981–1990) there were approximately 16,000 publications focused on obesity [1], while in the last year more than 20,000 articles on obesity were published. Interestingly, twin studies showed there is a strong genetic component to obesity [2, 3]. In accordance with this data, Claude Bouchard and colleagues generated a yearly human obesity genetic map between 1996 and 2005 [4], with the last report listing 176 human obesity cases relating to single-gene mutations in 11 different genes, and 50 loci relating to Mendelian syndromes relevant to human obesity that mapped to a specific genomic region. Their mouse obesity gene map identified 248 genes that, when mutated or overexpressed as transgenes in the mouse, resulted in phenotypes affecting body weight or adiposity. Many of these genes are expressed in the hypothalamus, and one of the earliest discovered genes, having a strong genetic association to human obesity, was the hypothalamic *MC4 receptor* (*MC4R*). Mc4r is inhibited by the orexigenic peptide Agrp and stimulated by the anorexic agonists α- and β-Msh [5]. The α- and β-Msh peptide hormones originate from the precursor protein Pomc, and humans with a mutated *POMC* gene have severe obesity [6]. Enormous resources have been put into MC4R research, as it was shown that synthetic and selective agonists and antagonists are very effective in reducing food intake and bodyweight in animal feeding models [7, 8]. To

date, this has not resulted in any promising drugs, mainly due to the complexity of the action of the melanocortin system, including effects on the cardiovascular system, creating risks for severe side effects.

Genome-wide association (GWAS) studies have transformed our knowledge of which genes are most important for obesity. The first gene that was strongly associated with obesity was fat mass- and obesity-associated (*FTO*) [9]. The *FTO* gene is expressed in many tissues of the body but, interestingly, it is highly expressed in hypothalamic feeding regions [10]. The molecular mechanism of how gene variants of *FTO* may cause obesity is still unknown, it has been suggested that it affects food intake, as carriers of the risk allele tend to choose high energy and more palatable food [3, 11, 12]. *Fto* knockout models and mutant mice point towards a role for Fto in energy homeostasis, metabolism and adipogenesis, and mutant mice have clearly decreased body fat mass [13, 14]. Moreover, transgenic mice that overexpress Fto have a higher body weight, and hypothalamic Fto expression is regulated in several animal feeding models [14].

Soon after the discovery of *FTO* as an obesity gene, six additional loci were found to be associated with obesity [15]. Only one of these genes, *SH2B1*, had any prior evidence linking it to obesity; knocking out *Sh2b1* in mice resulted in obesity, hyperglycemia, insulin resistance, and glucose intolerance. Thorleifsson and collegues identified new sequence variants at seven loci associated with obesity in GWAS of more than 30,000 individuals [16]. In total, 29 variants in 11 chromosomal regions reached a genome-wide significance threshold. These associations included the brain-derived neurotrophic factor (BDNF) gene, which was previously associated with both BMI and eating disorders [17, 18].

Meta-analysis of several GWAS, and other association studies involving almost 250,000 individuals, showed association with 14 known obesity susceptibility loci, while an additional 18 new loci were associated with BMI [19]. It is estimated that, similar to the 37 genes linked to these 32 loci, more than 250 additional common variant loci remain to be discovered that will have effects on BMI. It should be mentioned that GWAS studies identify genomic regions, rather than individual genes, and several of the genes currently linked to obesity by associated SNPs may later prove not be a causative agent in obesity. To date, only 8 of the 32 loci identified by Speliotes et al. [19] are strongly linked to an adjacent common missense SNP, and only 15 of the loci contain genes that can be biologically linked to obesity. This lack of a causative link is a result of the limited understanding of the biology behind obesity and weight regulation, which calls for a comprehensive investigation of these obesity-associated genes.

Among the 32 loci that have emerged as currently the most important ones for BMI [19], MC4R and POMC have clearly understood physiological roles, and their functions are well reviewed. Moreover, the research on BDNF and FTO is very intense, and there are several recent reviews addressing what is known about these genes. Here, we have focused on what is known about a select group of the additional 33 obesity-linked genes (*GIPR*, *GPRC5B*, *SH2B1*, *HMGCR*, *PRKD1*, *TUB*, *ZNF608*, *TFAP2B*, *KCTD15*, *SEC16B*, and *MTCH2*) and used the growing number of databases to search for information that can shed light into the functional role of these genes. These genes were chosen because further information beyond the GWAS studies was available, yielding possible evidences for their involvement in obesity. We have in particular taken advantage of model organisms that are distant to humans, as we find that 24 of the 33 newly discovered genes are well conserved in most bilateral species, including *D. melanogaster* and *C. elegans* (Table 1).

A subgroup of obesity-linked genes

In this review, we introduce a select subgroup of obesity-linked genes, including what is known about their domain structure and possible functions in distant model organisms. We also present some possible interactions between obesity-linked genes and how this interaction may regulate homeostasis.

Gastric inhibitory peptide receptor (GIPR): resistance to diet-induced obesity

GIPR is a G protein-coupled receptor (GPCR) belonging to the secretin-family that includes receptors for the peptides GLP1, glucagon, PACAP, VIP and secretin. The GIPR ligand, gastric inhibitory peptide (GIP), is released from intestinal K cells and potentiates glucose-stimulated insulin secretion by elevating cAMP levels, inhibiting the K_{ATP} channel and increasing intracellular Ca^{2+} [20]. GIPR is highly expressed in the pancreas, but is also found in a wide range of peripheral tissues. Studies using *Gipr* null mice established the importance of Gipr signalling to maintain glucose homeostasis and regulate lipid metabolism [21, 22]. In response to orally administered glucose, mice lacking Gipr signalling exhibited mild glucose intolerance and reduced levels of glucose-stimulated insulin secretion [21, 23]. However, mice given an intraperitoneal glucose challenge displayed normal fasting glucose levels and a normal glycemic index [23]. These findings suggest that GIPR stimulates insulin release, indicating that GIP functions as an incretin hormone. One potential explanation for the mild glucose intolerance observed in mice with a single incretin receptor mutation, either GIPR or the related glucagon-like peptide receptor (GLPR), is that loss of one incretin

Table 1 Evolutionary conservation of obesity-linked genes in major model organisms

Human	*G. gallus*	*D. rerio*	*D. melanogaster*	*C. elegans*	*S. cerevisiae*	*D. discoideum*
Receptors, ligands and signal transduction						
CADM2	CADM2	Cadm2a, cadm2b				
GIPR	GIPR	gipr				
GPRC5B	GPRC5B	gprc5b	boss			
LRP1B	LRP1B	lrp1b	Lrp1	lrp-2		
NEGR1	NEGR1	Negr1	CG11320			
NRXN3	NRXN3	Nrxn3	Nrx-1	nrx-1		
SH2B1	SH2B1	sh2b	Lnk			
Enzymes						
FANCL	FANCL	fancl	Fancl			fncl
GNPDA2	GNPDA2	gnpda2	Oscillin	T03F6.3		gnpda1
HMGCR	HMGCR	hmgcra	Hmgcr	F08F8.2	HMG1	hmgB
MAP2K5	MAP2K5	map2k5				
NUDT3	NUDT3	nudt3	Aps	Y92H12BL.5	DDP1	
PRKD1	PRKD1	prkd1	PKD	dkf-2		
QPCTL	QPCTL	qpctl	CG5976	H27A22.1	YFR018C	qpct
TNNI3K	TNNI3K	tnni3k		C24A1.3		
Transporters						
MTCH2	MTCH2	mtch2	Mtch	F43E2.7		
DNAJC27	DNAJC277	rbj				
SLC39A8	SLC39A8	slc39a8				
Protein processes						
MTIF3	MTIF3	mtif3	CG13163			
RPL27A	RPL27A	rpl27a	RpL27A	Y37E3.8	RPL28	rpl27a
SEC16B	SEC16B	sec16b	Sec16	F13B9.1	Sec16	
Transcription factor						
ETV5	ETV5	etv5	ETS96B			
HMGA1	HMGA1	hmga1				
TFAP2B	TFAP2B	tfap2b	AP-2	aptf-1		
TUB	TUB	LOC568677	king-tubby	tub-1		
ZNF608	ZNF608	znf608	sbb			
Unkown function						
FAIM2	FAIM2	faim2		xbx-6		
KCTD15	KCTD15	kctd15	CG10440			
LINGO2	LINGO2	lingo2				
POC5	C5orf37	POC5				
PTBP2	PTPB2	Ptpb2	heph	ptb-1		
TMEM18	TMEM18	tmem18	CG30051			
TMEM160		tmem160				

Using NCBI HomoloGene, EMBL-EBI databases (http://www.ebi.ac.uk/) and protein sequence similarity searches (http://hmmer.janelia.org/search/phmmer,UniProtKB), orthologues for the obesity-linked genes in a variety of model organisms were determined. Genes are grouped according to molecular function

receptor invokes upregulation of compensatory factors, particularly enhanced activity of the remaining incretin [24, 25]. Support for this hypothesis is derived from observations that *Glpr* null mice exhibit increased circulating levels of Gip, as well as enhanced sensitivity to the insulinotropic actions of Gip [26].

Gip has no direct effect on food intake or satiety, yet *Gipr* null mice exhibited resistance to diet-induced obesity, even after months of high-fat feeding, and when crossed with obese (ob/ob) mice, ob/ob diet-induced obesity was attenuated [27]. How could this be explained? It was observed that administration of the GIPR agonist

[D-Ala2]GIP increased plasma levels of the plasma adipokine resistin in wild-type mice. This observation confirmed that GIPR signalling is an essential component of the adipocyte response to chronic nutritional excess [27]. Observations linking GIP action to the modulation of resistin and control of energy expenditure explain the divergent effects of GIP action on pancreatic β cells and adipocytes. Whereas loss of GIPR function in β cells impairs the adaptive islet response to metabolic stress, the potential deleterious effects arising from loss of GIPR signalling, and thus impaired insulin secretion, is offset by the persistence of insulin sensitivity, likely arising through a combination of reduced resistin, decreased adipose tissue mass, and increased energy expenditure [28].

Recently, it was observed that the microRNA miR-642 is upregulated during adipogenic differentiation, and in 19 out of 20 obese subjects is highly expressed in fat depots [29, 30]. This is interesting because miR-642 is positioned within intron seven of *GIPR*, and may share the same promoter [29], leading to the possibility that at least some of the obese phenotypes associated with *GIPR* may be due to misregulation of miR-642.

G-protein coupled receptor family C group 5 member B (GPRC5B): possible glucose sensor

GPRC5 is a member of the family-C GPCRs, this family includes mGluRs, calcium-sensing receptors (CaRs), type B γ-aminobutyric acid receptors (GABABRs), putative pheromone receptors (V2Rs) and taste receptors (T1Rs), as well as *Drosophila* bride of sevenless (BOSS) [31, 32]. Within family-C, GPRC5B belongs to the retinoic acid inducible gene (RAIG) subgroup, consisting of at least four orphan receptors [31–35].

Although little is known about GPRC5B signalling in mammals, it has been observed that *Gprc5b* knockout mice had developmental and behavioural defects. Approximately 30 % of homozygous *Gprc5b* knockout mice died at postnatal day 0, while a further 50 % died by 4 weeks of age [36]. The 20 % who survived into adulthood had behavioural defects, including reduced activity before light onset, and in the open field test, travelled less distance and spent more time in the centre of the field, compared to control mice [36].

A recent report on *Drosophila* points to a possible function of GPRC5B in glucose metabolism. The *Drosophila* GPRC5B homologue, BOSS, was first identified as a ligand for the tyrosine kinase Sevenless, involved in eye differentiation [37]. However, as with other GPRC5B homologues, until recently the physiological function of BOSS as a GPCR was completely unknown. It was observed that *boss*-deficient flies are reduced in size, suggesting that it might be required for cell growth, cell size and survival [38]. Further analysis determined that BOSS is expressed in a *Drosophila* nutrient-sensing tissue, the fat body, and is a glucose-responding GPCR required for the homeostatic regulation of glucose and lipids. *Boss* mutants have downregulated insulin signalling activity, demonstrating that BOSS has a critical function regulating energy homeostasis [38, 39]. This finding represents an example of a glucose-responding GPRC5B homologue in a model organism. Since GPRC5B is conserved from *Drosophila* to humans, this provides evidence that GPRC5B maybe a nutrient-sensing GPCR.

SH2B adapter protein 1 (SH2B1): signals in the insulin pathway

Mammalian *SH2B adapter protein 1* (*SH2B1*) belongs to a family of adapter proteins known to regulate several different tyrosine kinases, including the receptors for insulin and insulin-like growth factor-1 [40–44]. As a result of these interactions, SH2B proteins are known to function during glucose homeostasis, energy metabolism and reproduction, and in humans, mutations in SH2B1 are associated with metabolic syndrome [45–49]. Furthermore, *Sh2b1* deletions in mice produce neonatal growth retardation and infertility possibly due to impaired responses to growth hormone or Igf-1 signalling [50]. *Sh2b1* null mice significantly increase their body mass and develop obesity as a result of impaired hypothalamic leptin signalling [47]. Intriguingly, neuronal restoration of Sh2b1 expression rescued leptin resistance, as well as the obesity phenotype, suggesting that Sh2b1 is involved in regulating energy balance and body weight by enhancing leptin sensitivity. In this same study, it was observed that loss of Sh2b1 in peripheral tissues induced insulin sensitivity regardless of body mass [47]. This result indicates that Sh2b1 regulates insulin signalling in peripheral tissues.

How might SH2B1 function to increase insulin signalling? Recent evidence demonstrated that SH2B1 physically interacts with two components of the insulin-signalling pathway, the insulin receptor (INSR) and insulin receptor substrate 1 (IRS1), and that this interaction is necessary to increase phosphoinositide 3-kinase (PI3K) activation downstream of IRS1 [40]. SH2B1 interaction with IRS1 inhibits dephosphorylation of IRS1 by protein tyrosine phosphotase 1B (PTP1B), leading to increased PI3K activation (Fig. 1). Furthermore, it was shown that SH2B1 interaction with INSR may increase INSR auto-phosphorylation, thus increasing INSR signalling.

The fact that SH2B1 signals in the insulin pathway was substantiated in *Drosophila melanogaster*. The *Drosophila* genome contains a single SH2B homologue, known as *Lnk*, which shares a similar domain structure to its mammalian counterparts, including the highly conserved c-Cbl binding

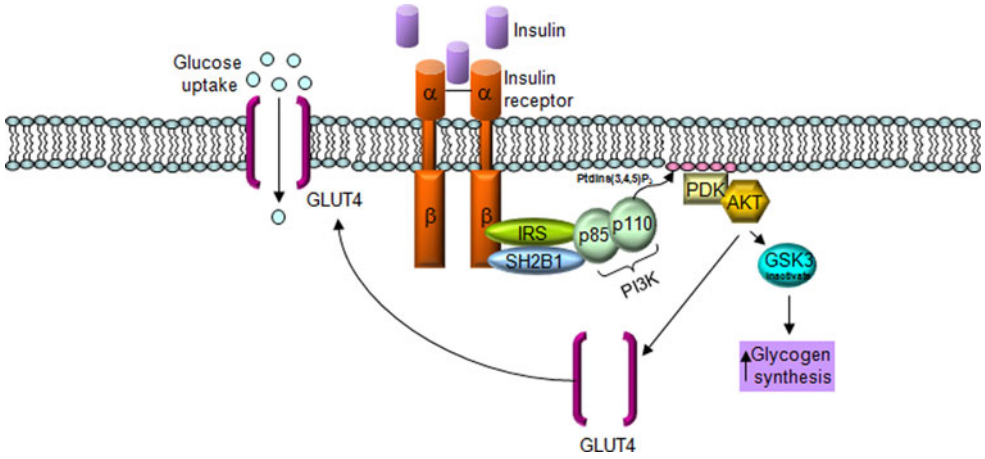


Fig. 1 SH2B1 signals in the insulin pathway. Insulin activates the insulin receptor (*INSR*), leading to INSR autophosphorylation and activation. Active INSR binds to and phosphorylates insulin receptor substrate-1 (*IRS1*), leading to activation of the downstream PI3K signalling pathway. Phosphorylated IRS1 is recognized and bound by SH2B1, inhibiting IRS1 dephosphorylation by protein tyrosine phosphatase 1B (*PTP1B*), thus prolonging insulin signalling pathway activation. Glucose transporter type 4 (*GLUT4*), Phosphoinositol 3-kinase (*PI3K*), phosphoinositide-3-kinase, regulatory subunit 1 (*p110*), phosphoinositide-3-kinase, regulatory subunit 2 (*p85*), Pyruvate dehydrogenase kinase (*PDK*), v-akt murine thymoma viral oncogene (*AKT*), Glycogen synthase kinase 3 (*GSK3*)

motif. In *Drosophila*, loss-of function *Lnk* mutations produce phenotypes reminiscent of reduced insulin and insulin-like growth factor-1 (IIS) signalling, including growth reduction, developmental delay and female sterility [46, 51]. Furthermore, classical genetic epistasis analysis established that, upon *Drosophila* insulin receptor (InR) activation during cell growth and division, Lnk signals in parallel with the *Drosophila* insulin receptor substrate, known as Chico, to activate PI3K [52].

Mutations reducing insulin/IGF-like (IIS) activity in multiple model organisms, including *C. elegans*, *Drosophila* and mouse, is known to increase lifespan. In *Drosophila*, recent studies showed that loss of *Lnk* increased lifespan, as well as improved survival during oxidative stress and starvation [46, 51, 52]. Furthermore, *Lnk* loss-of-function results in increased stored energy reserves associated with transcriptional changes in genes involved in both lipid and carbohydrate metabolism. Finally, in *Drosophila,* genetic analysis indicates that *Lnk* is itself a direct target for transcriptional regulation by the dFoxo transcription factor, indicating that *Lnk* transcription is regulated by insulin signalling [51].

3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR): regulates cholesterol production

3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) is a transmembrane protein located on the smooth endoplasmic reticulum (SER), necessary to catalyze the production of mevalonate, the rate-limiting step during cholesterol biosynthesis in mammals [53, 54]. Tight control of cholesterol biosynthesis is physiologically critical, as overproduction can induce a predisposition to atherosclerosis [55]. In mammals, HMGCR production is regulated by cholesterol levels in a negative feedback look, while in mammals and *Drosophila,* HMGCR regulation is linked with levels of insulin, which strongly stimulates its production [56–59]. Insulin regulation of HMGCR involves a family of helix-loop-helix transcription factors, known as sterol response element binding proteins (SREBP) [60–63].

In mammals, mevalonate synthesized by HMGCR is required to produce cholesterol, which is used as a precursor for corticoid production by the adrenal glands or androgen production by the gonads [64, 65]. In insects, cholesterol production is not generated downstream of mevalonate, but in the *Drosophila* corpus allatum, mevalonate is used to synthesize Juvenile Hormone (JH), in response to insulin signalling from the *Pars intercerebralis* [59, 66, 67]. In adult flies, JH is necessary to regulate sexual dimorphic behaviour, such as variations in locomotor activity between males and females [59]. HMGCR also regulates adult body size in response to insulin signalling in *Drosophila* [59, 67].

Protein kinase D1 (PRKD1): regulates insulin secretion

PRKD1 is a member of the serine/threonine-protein kinase family. Along with the kinase domain, PRKD1 is predicted to contain a pleckstrin homology (PH) domain and two C1 domains. PH domains are known to possess multiple functions, including binding to inositol phosphates, as well as being involved in various protein–protein interactions

[68–70], while C1 domains bind to the second messenger diacylglycerol (DAG) [71]. Protein kinase D1 is implicated in cancer, possibly through regulation of angiogenesis [72], and has also been shown to be involved in Toll-like receptor signalling in the immune response; specifically, it is necessary for the activity of the adaptor protein Myd88 [73].

It was previously determined that PKD signals downstream of a Gq protein-coupled receptor (GqPCR) activated by acetylcholine [74]. When acetylcholine binds to the GqPCR M3-muscarinic receptor (M3R), it activates phospholipase C (PLC), leading the production of diacylglycerol (DAG), which in turn activates PKD (Fig. 2) [75]. It has been demonstrated that glucose passing through the SLC2A2 (also known as GLUT2) channel also leads to the production of DAG [76, 77]. DAG-activated PKD translocates to the trans-Golgi network (TGN) where it is necessary for vesicle membrane fusion. It was observed that this was also the case for insulin secretion; blocking PKD in INS-1 cells inhibited insulin secretion, but not insulin production [78]. In these same cells, it has been reported that loss of p38δ lead to increased insulin secretion, and that this increase in insulin secretion was due to hyperactivation of PKD [78]. Interestingly, a link between protein kinase D and insulin signalling was reported in *C. elegans*. Loss of the *C. elegans PRKD1* orthologue, *dkf-2*, increases the worms lifespan by as much as 40 % over wild-type [79]. This increase in lifespan can be rescued in worms that also lack the *C. elegans* FOXO orthologue, *daf-16*. FOXO translocation to the nucleus is inhibited by insulin signalling, and the *C. elegans* result is further indication that PKD is regulating insulin signalling.

Tubby (TUB): affects late-onset obesity

The mouse *tubby* mutation is the cause of maturity-onset obesity, insulin resistance and sensory deficits [80]. In contrast to the rapid juvenile-onset weight gain seen in diabetes (db) and obese (ob) mice, tubby mice become obese gradually as they age. This phenotype strongly resembles late-onset obesity observed in maturing humans. In the end, in *tubby* mice, excessive deposition of adipose tissue culminates in a twofold increase of body weight. Although this, along with the insulin resistance and sensory deficit phenotypes, indicate a vital role for tubby proteins, no molecular function has yet been attributed to this family of proteins [81]. TUB belongs to the tubby-like proteins, or TULPs, which are found in both the plant and animal kingdoms.

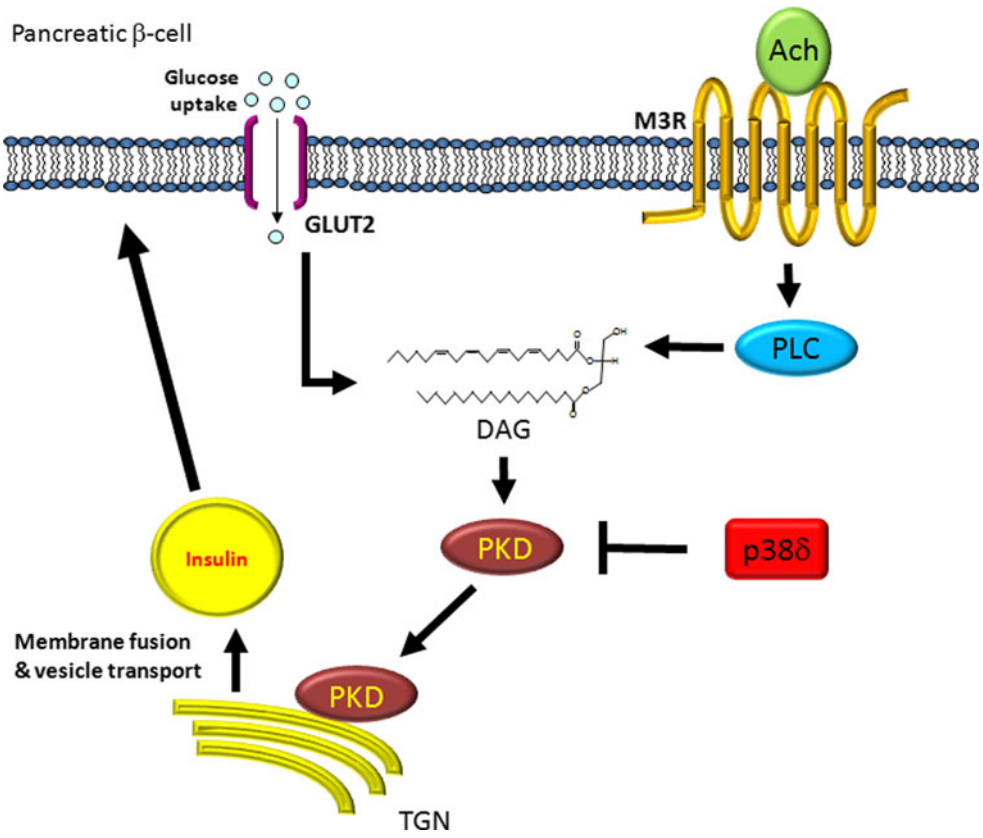


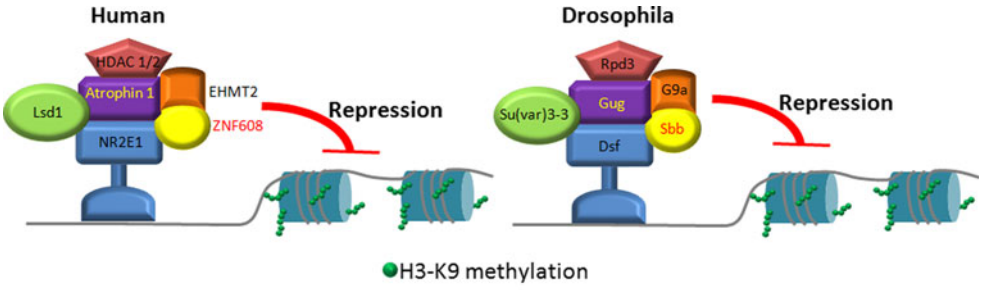
Fig. 2 Protein kinase D (*PKD*) is necessary for insuln secretion. Acetylcholine (*Ach*) and glucose both induce pathways leading to PKD activation. Ach binds to and activates the GPCR M3R, leading to induction of phospholipase C (*PLC*), or glucose passing through the glucose transporter (*GLUT*), leading to the production of diacylglycerol (*DAG*), which in turn activates PKD. Activated PKD interacts with the trans-Golgi network (*TGN*) to regulate the production and transportation of vesicles, including those which contain insulin, to the plasma membrane. PKD is inhibited by the MAP kinase p38δ. *M3R* muscarinic acetylcholine receptor M3

The N-terminal portion of TULP protein is not very conserved, but mammalian TUB contains a nuclear localisation signal and may have transcriptional-activation activity. The C-terminal residues of TULP family members are highly conserved, containing a cysteine residue that might play an important role in the normal functioning of these proteins. This domain is arranged as a 12-stranded, all anti-parallel, closed beta-barrel that surrounds a central alpha helix that forms most of the hydrophobic core. Structural analyses suggest that TULPs constitute a unique family of bipartite transcription factors [81]. In *Drosophila*, the TULP orthologue, known as *king-tubby*, is highly expressed in the developing nervous system [82]. In *C. elegans*, loss of *tub-1*, the worm orthologue of *TUB*, causes an increase in the storage of triglycerides and leads to a significant increase in life span [83]. While the increase in life span was rescued by removing the *daf-16*, the *C. elegans* FOXO homologue, the increase in lipid storage required TUB-1 interactions with the Rab GTPase-activating protein RBG-3, the orthologue of human TBC1D5. The results in *C. elegans* could mean that, similar to PRKD1, TUB is involved in regulating insulin secretion. On the other hand, this same group went on to show that TUB-1 and RBG-3 signal through RAB7 to regulate lipid storage in *C. elegans* [84]. In mammalian cell lines, TBC1D5 and Rab7 are both involved in regulating autophagy, and recently Rab7 was shown to signal downstream of insulin-like growth factor 1 (IGF1) to regulate autophagy in cultured neuronal cells [85, 86]. Considering the expression pattern of king-tubby in *Drosophila*, TUB may signal in neuronal cells during development to regulate autophagy.

Zinc finger protein 608 (ZNF608): regulates histone methylation for gene repression

Although nothing is known about possible molecular mechanisms of ZNF608 function, the *Drosophila* homologue of ZNF608, known as *scribbler* (*sbb*), is involved in the resistance to starvation, with *sbb* mutants being more susceptible to starvation [87]. Also, in *Drosophila*, Sbb bound directly to Grunge [known as arginine-glutamic acid dipeptide (RE) repeats, RERE, in humans], and together with the nuclear hormone receptor Tailless (Tll), known as TLX or NR2E1 in humans, they repressed the expression of the GAP gene *knirps* [88]. Finally, in this same study, it was shown that human ZNF608 and a closely related RERE protein, known as Antropin-1, directly interact, showing a conservation of function between the *Drosophila* and human proteins.

RERE can recruit HDAC1 and HDAC2 and the histone H3-K9 methyltransferase G9a [89] (Fig. 3). By directing the activities of HDAC1/2 and G9a, RERE catalyzes sequential molecular events, first causing deacetylation of H3-K9 and then allowing the deacetylated residue to be methylated by G9a [89]. As a result, chromatin regions where RERE binds are converted to compact structures, favouring gene silencing (Fig. 3). In *Drosophila*, the Atrophin-Rpd3 (HDAC1/2)-G9a complex represses the EGFR signalling pathway. During metamorphosis, wing vein formation is initiated by activated EGFR [90]. A mutation of *Atro*, or reduced expression of Atro using RNAi, results in ectopic vein formation in the intervein regions [89, 91, 92]. This ectopic wing vein phenotype is enhanced when *G9a* or *Rpd3* is also mutated [89]. These



Human	Drosophila	Function
NR2E1	dissatisfaction (dsf)	Nuclear orphan receptor
Atrophin 1	Grunge	Transcriptional co-repressor/scaffolding protein
ZNF608	scribbler (sbb)	Unknown
EHMT2	G9a	Histone methyltransferase
HDAC 1/2	Rpd3	Histone deacetylase
Lsd1	Su(var)3-3	Histone demethylase

Fig. 3 ZNF608 functions in the atrophin transcriptional repression machinery. A model depicting the actions of the Atrophin-protein complex on chromosomes. Atrophin-1, or Grunge in *Drosophila*, recognizes nuclear hormone receptors and forms a complex with histone deacetylaces (HDACs), histone demethylase (Lsd1 in humans, Su(var)3-3 in *Drosophila*), histone methyltranserfase (EHMT2 in humans, G9a in *Drosophila*) and ZNF608 in humans or Scribbler in *Drosophila*. This complex induces methylation of histone H3 leading to transcriptional repression

genetic data indicate that Atro, G9a and Rpd3 act together to repress wing vein formation, perhaps by antagonizing the activities of EGFR.

Possible interactions between obesity-linked genes

TFAP2B and KCTD15: potential interactions in adipocytes

Transcription factor AP-2beta (*TFAP2B*) is a member of the AP-2 family of transcription factors, key regulators of various developmental processes [93–95]. AP-2 family members can homo- and heterodimerize through a highly conserved C-terminal helix-span-helix motif and bind to DNA by means of a basic domain immediately N-terminal of the dimerization domain. *Potassium channel tetramerisation domain containing 15* (*KCTD15*) belongs to a family of potassium channel tetramerization domain-containing proteins. Like all KCTD family members, KCTD15 contains a Broad complex, Tramtrack and Bric-a-brac (BTB) domain. To date, only one study on KCTD15 has been performed to elucidate its molecular function. In zebrafish embryos, Kctd15 functions to inhibit the Wnt signalling pathway, in order to restrict neural crest formation, though the exact mechanism of this inhibition is unknown [96]. Interestingly, in *Drosophila*, the Wnt responsive gene *hedgehog* plays a role in adipogenesis and in mice is involved in the determination of brown and white adipose tissue [97].

A large-scale yeast two-hybrid screen was carried out in *Drosophila*, using almost the entire proteome, in an attempt to find all possible protein–protein interactions [98]. In this screen, the *Drosophila* homologues of *TFAP2B* (*AP-2* in *Drosophila*) and *KCTD15* (*CG10440* in *Drosophila*) had a strong interaction. Furthermore, mRNA in situ analysis in *Drosophila* embryos demonstrated that *AP-2* and *CG10440* co-express in the developing brain [99, 100]. These two results, and the fact that both *TFAP2B* and *KCTD15* are linked to obesity in multiple genome-wide association studies, indicate a possible physical interaction in vivo. It must be mentioned that yeast two-hybrid screens are not definitive evidence for an interaction between two proteins, and further experiments need to be performed to ascertain whether or not AP-2 and CG10440 proteins actually interact.

Taking what is known about AP-2 and KCTD family members, we propose the following model for a possible TFAP2B and KDTD15 interaction (Fig. 4). In adipocytes, AP-2β is a negative regulator of insulin receptor substrate-1 (IRS1) expression [93]. IRS1 links signalling from the insulin receptor (INSR) to the phosphoinositol 3-kinase (PI3K) and Akt pathways, and reduced IRS1 expression is one of the key molecular events involved in insulin resistance [101]. Also in adipocytes, Ap2α function was inhibited by sumoylation, requiring the SUMO-conjugating enzyme Ubiquitin carrier protein 9 (UBC9) [102]. In this same study, UBC9 was also shown to sumoylate AP-2β. KCTD5 and 11 interact with cullin E3 ligase (CUL3), and this complex then binds to E2 ubiquitin ligase [103–105]. Though no interaction with the sumoylation complex has been shown, using SUMOsp 2.0 Sumoylation Site Prediction [106, 107], we found a conserved sumoylation site in all KCTD family members. This leads to the possibility that KCTD family members are able to interact with the sumoylation apparatus. Furthermore, in a yeast two-hybrid screen, the *Drosophila* KCTD15 homolog, CG10440, was shown to interact directly with Lesswright (Lwr), the *Drosophila* UBC9 homolog [98].

In our model (Fig. 4), high glucose levels would potentially induce UBC9 to interact and sumoylate KCTD15, and this complex could then bind to dimers of AP-2β, leading to their sumoylation and inhibition. AP-2β inhibition would allow for increased IRS1 transcription, and thus increased insulin sensitivity. Insulin signalling, in response to increased glucose levels, would inhibit UCB9 activation, leading to AP-2β desumoylation and activation. Activated AP-2β would inhibit IRS1 transcription, thus inducing insulin resistance. It has been published that AP-2α interacts with another KCTD family member, KCTD1. Interestingly, the domain in AP-2α required for interactions with KCTD1 is conserved in AP-2β and in *Drosophila* AP-2 [108].

SEC16B and MTCH2 may help to regulate Ca^{2+} stores

Intracellular Ca^{2+} is required for the proper regulation of multiple important processes within a cell, and the formation of a precise spatiotemporal Ca^{2+} signal depends on extensive cellular machinery. Recently, it became evident that there is a complex interplay between the endoplasmic reticulum (ER), a major storehouse for Ca^{2+}, and the mitochondria, to regulate not only cytoplasmic Ca^{2+} but also ER Ca^{2+} stores [109].

ER Ca^{2+} levels are regulated, in part, by the inositol 1,4,5-triphosphate receptor (IP3R) [110]. The amount of Ca^{2+} transferred from the ER to mitochondria not only depends on the activity of the IP3R but also on the distance between the mouth of the IP3R and voltage-dependent anion channels (VDAC), the major protein family involved in mitochondrial Ca^{2+} uptake. For this interaction to occur, the distance between the ER and mitochondria must be regulated, too close or too far and Ca^{2+} transfer is less efficient. This means that the cell needs a mechanism to maintain a stable distance between ER and mitochondrial membranes. Heat shock 70-kDa protein 9 (HSPA9), also

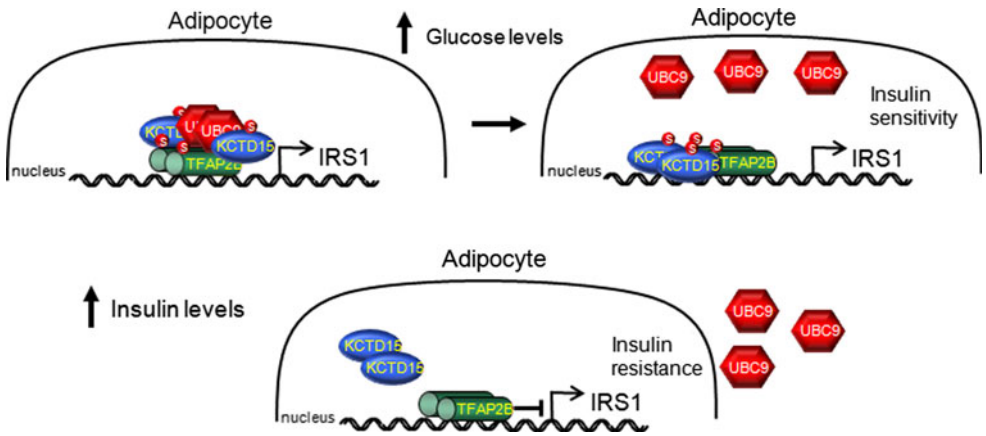


Fig. 4 *Transcription factor AP-2beta* (*TFAP2B*) and *potassium channel tetramerization domain containing 15* (*KCTD15*) may regulate insulin receptor substrate 1 (IRS1) expression. Model predicting a possible interaction between AP-2β and KCTD15 in the regulation of IRS1. High glucose levels would activate the sumylation regulatory protein Ubiquitin carrier protein 9 (UBC9). UBC would then sumoylate KCTD15 and together they would bind to dimers of AP-2β. UBC9 sumoylation of AP-2β would inhibit its function, allowing for increased IRS1 transcription. Increased insulin signalling would feedback to release AP-2β inhibition and reduce IRS1 transcription

known as mortalin, is one protein implicated in bridging this gap. HSPA9 binds to both IP3R and VDAC family members, allowing for better transfer of Ca^{2+} (Fig. 5) [111, 112]. Although HSPA9 controls interactions between IP3R and VDACs, thus helping to regulate gap distance, it is not the major determinant of the ER–mitochondrial bridge [110, 112].

So what is regulating the gap distance? The ER protein Sec16 homolog B (SEC16B) and the mitochondrial protein mitochondrial carrier 2 (MTCH2) are both linked to obesity. The *S. cerevisiae* SEC16B homologue, known as Sec16, is a peripheral membrane protein necessary for ER-to-Golgi transport and cell viability [113]. Sec16-family members interact with the COPII machinery, including Sec23, Sec24 and Sec31, and are involved in the recruitment of the GTPase Sar1 to the ER exit sites (tER) (Fig. 5) [113–119].

MTCH2 shares homology with members of the mitochondrial carrier family, but unlike most family members, it is located on the mitochondrial outer membrane, where it co-localizes with a large protein complex, containing the apoptosis regulatory proteins tBID and BCL2-associated X protein (BAX) [120, 121] (Fig. 5). MTCH2 protein is required for tBID recruitment to the mitochondria and plays a essential role tBID-induced cell death [120].

In *Drosophila*, the COPII assembly proteins Sec16 and Sec24C have been shown to bind to the mitochondrial protein Tom40 [98]. Tom40 is the *Drosophila* homologue of mammalian translocase of outer mitochondrial membrane 40 (TOMM40), which is a channel-forming protein in the Tom40 complex, involved in mitochondrial protein import [122, 123]. In multiple organisms, the Tom40 complex and VDAC proteins directly interact to regulate

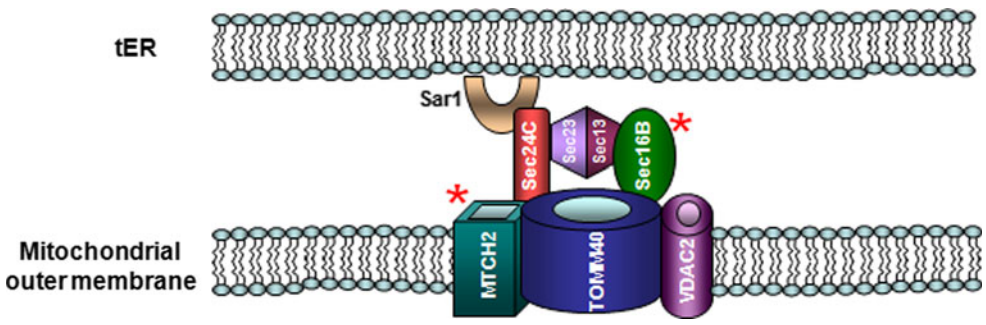


Fig. 5 Sec16 homolog B (SEC16B) and mitochondrial carrier 2 (MTCH2) may regulate ER and mitochondria gap distance. Depiction of interactions between the COPII apparatus at the tER and the translocase of the mitochondrial outer membrane 40 (Tom40) complex in the outer mitochondrial membrane. Interactions between the COPII machinery and the Tom40 complex would help regulate protein transit across the mitochondrial membrane, as well as maintain the correct distance between ER and mitochondrial membranes. This would, in turn, control the transfer of Ca^{2+} between the ER and mitochondria, which is used as a sensor for the induction of apoptosis. SEC24 family, member C (*Sec24C*), voltage-dependent anion channel 2 (*VDAC2*)

each other's functions [124–127]. It is postulated that MTCH2 may also interact with the Tom40 complex [128].

We suggest the following model for a SEC16B and MTCH2 interaction in regulating cellular homeostasis (Fig. 5). Tomm40 binding to SEC16B and SEC24C at the tER would facilitate mitochondrial protein import. It would also, along with HSPA9, help to anchor mitochondria close the ER, allowing calcium to pass out of the ER, via the IP3R, and into the mitochondria through VDAC. Mitochondrial calcium overload is a major inducer of apoptosis [129, 130], and would trigger MTCH2–BH3 interacting domain death agonist (tBid) interactions. In this way, the cell closely links ER and mitochondrial functions, necessary for the proper regulation of cellular Ca^{2+} levels. Disruption of this Ca^{2+} homeostasis would induce apoptosis. Interestingly, in *C. elegans*, TOMM-40, TOMM-20 and TOMM-22 are necessary for proper insulin secretion [131]. If SEC16B and MTCH2 interact with the TOMM complex in humans, they could also be involved in regulating insulin secretion.

Future perspectives

Current estimates suggest that there could be at least 250 genetic loci in humans important for the regulation of obesity [19], while the number of pathways or networks that these obesity genes participate in is likely to be much lower. Tremendous progress has been made recently in network biology, protein cooperatives, or modules displaying interaction between proteins, i.e. interactome networks. Many of these networks are highly conserved biological pathways throughout evolution. We show here that about half of the human obesity genes are highly evolutionary conserved and likely to be present in most vertebrates, and in some cases most bilateral species.

These obesity genes are not equally distributed through protein classes when compared with the rest of the genome. We see that genes involved in cell signalling, such as receptors (9 genes), ligands (2 genes) and signal transduction molecules (1 gene), corresponding to about one-third of the obesity associated genes, are significantly enriched ($p \leq 0.01$; hypergeometric test) when compared to the whole human genome, where about 16 % of the genes are annotated to have comparable functions (source: http://www.uniprot.org, http://www.geneontology.org). Similarly, we also identify a potential enrichment for transcription factors according to UniProt and Gene ontology ($p \leq 0.02$; 14 % of the obesity genes, $\sim$4 % of genome). However, when compared with human transcription factors listed in a comprehensive study by Ravasi and colleagues ($\sim$10 % of the genome) [132] the enrichment was not validated ($p > 0.92$) and should be considered as uncertain. The 9 enzymes and 2 transporters found among the obesity genes are not more than expected by chance.

We identified new potentially important protein interactions using expression data from mouse and *Drosophila* (BioGPS, Flyatlas), and other data obtained from *Drosophila* (yeast-two hybrid, embryonic mRNA in situ), as well as searching homologies (NCBI homoloGene) and protein interaction maps (Interlog finder, http://www.interlogfinder.org). We show that TFAP2B is linked to KCTD15 and SEC16B is linked to MTCH2. For HMGCR, we see a conservation of expression and function, as it is necessary to make cholesterol-derived products involved in regulating homeostasis, corticoids in the case of mammals and Juvenile hormone (JH) in *Drosophila* adults. These findings show that studies using model organisms, such as *Drosophila melanogaster*, can provide a conservation of function that, if exploited, could help in our understanding of how these obesity-linked genes function to regulate the homeostatic system.

Moreover, it is interesting to identify which of the genes in each network are the most suitable against which to develop drugs. One classical feature of drug targets includes the ability of the protein to form a cavity that a small molecule can lock in, and in most cases block the activity of the proteins. Indeed, the 9 receptors (LRP1B, NRXN3, GPRC5B, NEGR1, GIPR, CADM2, MC4R, FAIM2 and LRRN6C) and 9 enzymes (QPCTL, HMGCR, GNPDA2, FANCL, NUDT3, PRKD1, TNNI3 K, MAP2K5 and FTO) that together constitute about half of the obesity genes arguably have this important drug target feature. Also, 13 of the genes (CADM2, FAIM2, GIPR, GPRC5B, HMGCR, LINGO2, LRP1B, MC4R, MTCH2,NRXN3, QPCTL, SLC39A8 and TMEM18) code for transmembrane proteins, a highly used class of proteins in drug development and likely to play key roles in conveying signals over the membrane (another classical feature of drug targets). Furthermore, 14 of the genes (MC4R, GIPR, MAP2K4, PRKD1, TNNI3K, LINGO2, NEGR1, CADM2, KCTD15, SH2B1, QPCTL, GPRC5B, LRP1B and NRXN3) share protein family or protein domains with known drug targets, and HMGCR is targeted by a number of anticholesteremic agents, such as Lovastatin [133]. Hence, there are enormous possibilities that a considerable part of the currently identified obesity-linked genes can be targeted by traditional types of drugs. The recent success of monoclonal antibodies has allowed for additional opportunities for rational target selection and high target specificity. Among the obesity genes, there are two coding for ligands (BDNF and POMC); such gene products are not traditional drug targets, but may become interesting providing that an antibody could reach the intended site of action. This is obviously complicated by the fact that these

ligands are expressed in the CNS. In general, while our understanding of the functional roles of obesity-linked genes is currently very vague, no doubt distant model species, interactomics and signalling pathway analysis represent an important way to better understand the functional diversity of the surprisingly high number of molecules seemingly important for human obesity.

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