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Ciliary dysfunction and obesity

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Obesity associates with increased health risks such as heart disease, stroke and diabetes. The steady rise in the obese population worldwide poses an increasing burden on health systems. Genetic factors contribute to the development of obesity, and the elucidation of their physiological functions helps to understand the cause, and improve the prevention, diagnosis and treatment for this disorder. Primary cilia are evolutionarily conserved organelles whose dysfunctions lead to human disorders now defined as ciliopathies. Human ciliopathies present pleiotropic and overlapping phenotypes that often include retinal degeneration, cystic renal anomalies and obesity. Increasing evidence implicates an intriguing involvement of cilia in lipid/energy homeostasis. Here we discuss recent studies in support of the key roles of ciliary genes in the development and pathology of obesity in various animal models. Genes affecting ciliary development and function may pose promising candidate underlying genetic factors that contribute to the development of common obesity.

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The development of obesity involves genetic factors

Obesity increases the risk of adverse health effects such as cardiac disease, diabetes and overall reduced life expectancy (1–3). With 37% of the current US population considered obese, and a projected 50% of the adult American population becoming obese by 2030 (4), obesity imposes profound socioeconomic burdens on healthcare systems.

Despite obvious contributions from non-genetic factors such as decreased physical activity and the increased intake of high-caloric food, twin-based studies provided strong evidence for genetic contributions to the body weight (5, 6) or body mass index (BMI) in both children and adults (7, 8). Studies on monogenic human syndromes that involve obesity such as Bardet–Biedl syndrome (BBS), and on obese animal models (9–12), have begun to elucidate the physiological basis and genetic factors that play a role in the development of obesity.

The physiological basis of obesity

Energy homeostasis maintains a delicate balance between caloric intake, energy storage and expenditure. An imbalance in energy homeostasis results in obesity in the form of either excess adipose tissues and/or increased adipocyte size. Several recent publications provide thorough reviews on physiological factors that are involved in energy homeostasis (13, 14). Here we highlight several key aspects of energy homeostasis that are relevant to animal model studies to be discussed in later sections (Fig. 1).

Leptin, adiponectin and insulin are examples of circulating hormones that affect satiety and caloric intake (15–17). Secreted by adipose tissues, the circulating plasma level of leptin correlates with the size and abundance of those tissues. Leptin

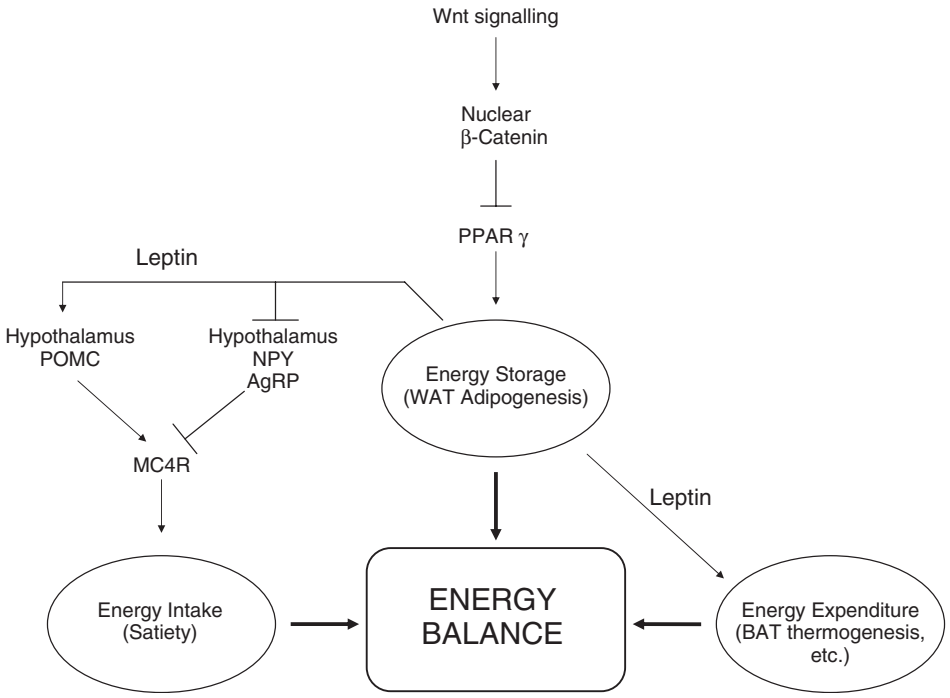

Fig. 1. Maintenance of energy homeostasis. Energy balance is maintained by multiple factors, including those that regulate satiety and adipogenesis. Outlined are key components to energy homeostasis that involve the primary cilia. Wnt signaling inhibits adipogenesis through the stabilization of β-catenin and its translocation to the nucleus to suppress peroxisome proliferator-activated receptor-γ accumulation. Leptin, secreted by white adipocyte tissue (WAT), plays a critical role in the hypothalamus to control the peptide secretion in pro-opiomelanocortin and neuropeptide Y/agouti-related peptide neurons. These peptides compete for the melanocortin-4 receptor to control satiety. Increased WAT populations secrete higher levels of leptin to suppress appetite. Decreased WAT levels attenuate leptin signaling resulting in hunger, increased caloric intake and decreased energy utilization.

controls appetite and regulates energy expenditure through the central nervous system (CNS) in mammals (18–20). In the CNS, the hypothalamus arcuate (ARC) neurons play a crucial role in satiety (21). In murine models, leptin and a leptin receptor (*Lepr*) isoform LepRb are implicated in the control of satiety through two distinct populations of ARC neurons: the pro-opiomelanocortin (POMC)-producing neurons that suppress appetite, and the neuropeptide Y (NPY) and agouti-related peptide (AgRP)-producing neurons that promote appetite (17, 22). In POMC neurons, LepRb, in response to leptin, triggers the nuclear translocation of signal transducer and activator of transcription 3 (STAT3), which activates the expression of POMC (23, 24). Through post-translational modification, this hormone precursor generates various peptides, one of which is the α-melanocyte-stimulating hormone (αMSH) that activates the melanocortin-4 receptors (MC4Rs) and suppresses appetite. In NPY/AgRP neurons, leptin signaling inhibits the secretion of the neuropeptide AgRP, which antagonizes αMSH activity (25, 26). Consistently, the specific inactivation of leptin receptors in POMC neurons resulted in severe obesity in the mouse (27), while the disruption of STAT3 activity in POMC neurons also results in mild forms of obesity (28, 29). Disrupting Mc4r in the mouse also resulted in adult-onset obesity that was associated with hyperphagia, hyperinsulinemia, and hyperglycemia (30–34).

Leptin also plays a part in the regulation of energy expenditure through thermogenesis in brown adipose tissue (BAT) (35). Once thought to be present only in neonates (36), the presence of metabolically active BAT is becoming increasingly evident in human adults (37–40). The toxin-induced specific ablation of BAT led to obesity in mouse models (41). In rodents, leptin was shown to increase the expression of Uncoupling Protein 1 (UCP1) (42), a mitochondrial protein specific for BAT, which promotes proton leakage and effectively disconnects the oxidation process from ATP generation (43). These studies thus implicate a role for leptin in thermogenesis and lipid homeostasis.

Adipose tissues store energy in the form of fat. The level of obesity directly correlates with the size and number of adipocytes. Human adipogenesis takes place mostly in childhood and adolescence. Adult-onset obesity is therefore generally attributed to excessive adipocyte storage, whereas

childhood obesity is often linked to misregulated adipogenesis that leads to increased adipocyte populations (44). The regulation of adipogenesis was examined using mesenchymal embryonic stem cell-derived precursors for adipocytes, such as the mouse 3T3-L1 cell line (45, 46). Pre-adipocytes are either maintained in a dormant state, proliferate, or terminally differentiate as adipocytes (47). The maintenance of the pre-adipocyte state requires the Wnt and Hedgehog (Hh) signaling pathways, whereas pro-adipogenic factors such as peroxisome proliferator-activated receptor-γ (PPARγ) and CCAAT-enhancer-binding protein-α promote terminal differentiation (48, 49). While the role of Hh signaling in adipogenesis remains slightly elusive and controversial (50), several studies implicated Wnt signaling as the major player in maintaining the balance of undifferentiated *vs* mature populations of adipocytes (51–53). In 3T3-L1 lines, the activation of the canonical Wnt signaling pathway led to reduced phosphorylation of β-catenin, which promoted its translocation to, and accumulation in the nucleus. Nuclear β-catenin repressed adipogenesis, at least in part through reducing the expression of PPARγ (54).

Ciliary development and function: association with obesity

Recent studies in animal models provide intriguing evidence that at least some aspects of the development of obesity involve the primary cilium, an evolutionarily conserved organelle that projects from the cell surface, and is now considered to be present in virtually all cell types (55). Here we discuss key studies that revealed roles for primary cilia in regulating satiety (56, 57) and adipogenesis (58–60), two important aspects of energy homeostasis.

Primary cilia and the role in development

Consisting of a 9 + 0 doublet microtubule structure, most primary cilia are non-motile. There are also rare instances of motile primary cilia such as the nodal cilia (61), and of transient 9 + 2 non-motile cilia such as the kinocilium during cochlear development (62). Enclosed with a unique complement of membrane proteins, the primary cilium is now viewed as the sensory antenna to coordinate cellular signaling during development (63–67).

The assembly and maintenance of ciliary structures requires the intraflagellar transport (IFT) system that carries out the bidirectional transport of protein/lipid cargos through motors and raft

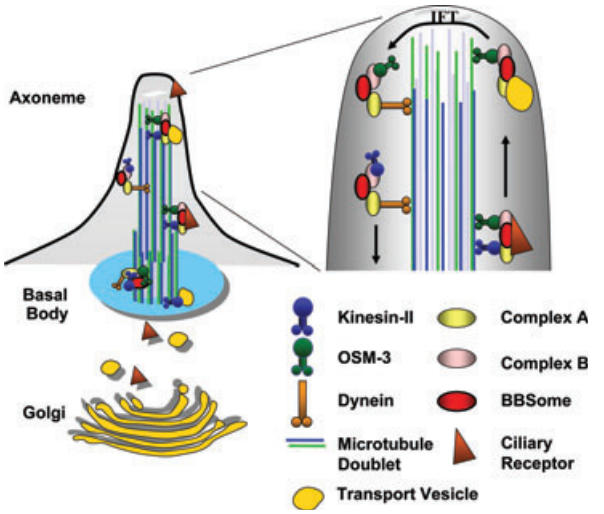

Fig. 2. Diagram of the primary cilium. Intraflagellar transport (IFT) motors and raft complexes coordinate the translocation of proteins from the basal body to the anterograde end of the primary cilium. IFT components can transport cargo both anterogradely and retrogradely along the cilium. The BBSome complex was proposed to play a critical role in the maintenance or coordination of IFT raft components at the basal body, and along the axoneme in the *C. elegans* primary cilium.

complexes. Two microtubule-based motors, a heterotrimeric kinesin-II complex (68) and dynein (69–71) drive the anterograde and retrograde transport, respectively. Multiple IFT components are assembled into two distinct raft complexes, complex A and complex B that shuttle between the base and tip of the cilium (Fig. 2) (72–74). More recently, another protein complex consisting of the BBS proteins, called the BBSome, was found to be required for ciliogenesis (75, 76) and proposed to maintain the association between the A and B complexes in the nematode *Caenorhabditis elegans* (*C. elegans*) (77–79). In addition to ciliogenesis and maintenance, IFT also plays an essential role in the transport of various signaling molecules in the cilium. For example, rhodopsin, a photoreceptor component, and Smoothened, a receptor in the Hh signaling pathway, are ciliary G protein-coupled receptors whose transport depends on IFT (58, 80).

The primary cilium plays a central role in the processing of multiple signaling pathways including that of Sonic Hedgehog (Shh), planar cell polarity (PCP), and Wnt (81–83). The loss of function in the raft components IFT172 and TG737, and a component of the kinesin-II motor, KIF3A, led to abnormal primary ciliary development and defective Hh signaling in the mouse (84). Further studies showed that not only were the Shh receptors, Smoothened and Patched1 localized to the primary cilium in an IFT-dependent

manner, but that their ciliary localization was also required for Shh signaling in the mouse and zebrafish (58, 85, 86). PCP signaling components including inversin and VANGL2 were found at the axoneme and basal body of cilia in mouse renal cells (87, 88). Furthermore, PCP phenotypes such as defective neural tube closure were later recapitulated in *Tg737* and *Bbs4* knockout mice, implicating a role for cilia in regulating PCP signaling (88, 89). Disturbance of Wnt signaling was first implicated in the *Kif3a* and *Ift88*^{orpk} knockout mice, which displayed an increased expression or mislocalization of β-catenin in the kidney and pancreas, respectively (90, 91). Recently, cilium-mediated regulation of canonical Wnt signaling was demonstrated more directly in mouse embryonic fibroblasts (MEF). When stimulated with Wnt3a, MEF derived from *Kif3a*$^{-/-}$ mouse displayed a higher level of β-catenin in cytoplasm as well as in the nucleus when compared to *Kif3a*$^{-/+}$, suggesting a role of the primary cilium in restricting the signaling response to Wnt (92).

Ciliopathies and obesity

Defective cilium biogenesis, IFT and localization of ciliary proteins are the leading causes for an emerging class of human diseases called ciliopathies. BBS is an example of a genetically heterogeneous ciliopathy with primary features that include photoreceptor degeneration, digit anomalies, cystic renal abnormalities, and obesity (93). Studies using various animal models, pioneered in *C. elegans*, demonstrated that BBS proteins localize to, and are required for the assembly and maintenance of the primary cilium (Fig. 2) (77, 94, 95). Another ciliopathy, Alström syndrome (AS), shares the clinical feature of obesity with BBS (96, 97). The obesity phenotype in BBS and AS patients sparked the interest in examining the molecular and cellular links between cilia and the development of obesity (98). Studies in various animal models provide supporting evidence for this hypothesis. Below we focus on recent studies that associate ciliary dysfunctions with satiety control and lipid homeostasis.

Regulation of satiety

Satiety signaling in the CNS dictates food intake. It was first observed that female *Bbs2*$^{-/-}$ mouse mutants displayed a significant weight increase and accumulation of abdominal fat by four months of age, despite being of a lower birth weight when compared to their heterozygous and wild-type littermates. These animals developed hyperphagic tendencies as early as 10 weeks of age; it was thus postulated that their obesity was linked to a dysregulation in satiety (99). More recently, Davenport et al. explored the relationship between the development of obesity and ciliary dysfunction by spatial and temporal-restricted genetic ablation of IFT components. Using a tamoxifen-inducible Cre recombinase system, selective disruption of the IFT raft component Tg737, or of the kinesin-II subunit Kif3a, between 8 and 12 weeks of age allowed the assessment of ciliary function in adult mice. Increases in body weight, organ size, serum leptin, glucose, and insulin levels were observed in both knockout models that were fed ad libitum. Restricted dietary intake prevented the increase in both weight and serum markers, further supporting a notion that the obesity and diabetic phenotypes were a consequence of satiety dysregulation

How does ciliary dysfunction affect satiety? Davenport et al. further observed that the genetic disruption of *Kif3a* in CNS neurons resulted in a similar, although slightly delayed weight gain. Furthermore, knockout of *Kif3a* in POMC neurons alone led to the loss of their primary cilium and a significant weight increase, indicating that the satiety defects were strongly associated with ciliary dysfunction in the POMC neurons. Weight gain caused by a global *Kif3a* disruption remained more severe; additional metabolic components perturbed by ciliary dysfunction therefore also contributed to the development of obesity in these mouse models (100).

Complementary studies by Seo et al. suggested that leptin resistance in BBS mutant mice was also associated with the ciliary dysfunction in the POMC neurons. *Bbs2*$^{-/-}$ mice had an increased leptin level when fed *ad libitum*, which could be prevented by pair-controlled feedings. Unlike their wild-type littermates, *Bbs2*$^{-/-}$, *Bbs4*$^{-/-}$ and *Bbs6*$^{-/-}$ mutants did not show decreased appetites or weight loss induced by leptin injections.

Blood-brain transport of leptin was normal in BBS animals (101). Therefore, the observed leptin resistance was attributed to attenuated leptin signaling in POMC neurons as they displayed a decreased level of phosphorylated STAT3, and *Pomc* transcripts. Intriguingly, a physical interaction between LepRb and BBS1 was observed in HEK293T cells; and in ARPE-19 cells, BBS1 was required for the trafficking of LepRb between the golgi and the cell surface (102).

These elegant experiments thus implicate a connection between ciliary dysfunction and obesity through, at least in part, satiety misregulation in the hypothalamus POMC neurons. Both KIF3A

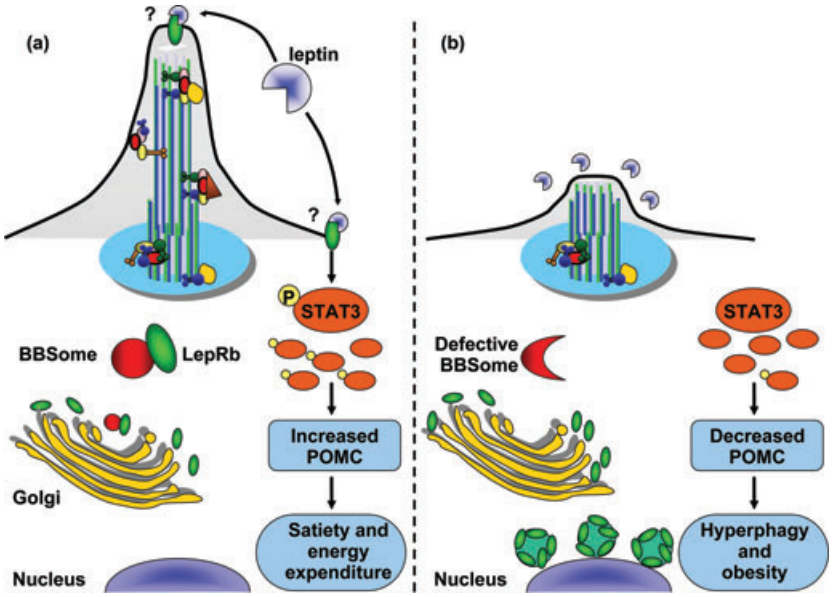

Fig. 3. Cilia participate in leptin signaling and satiety. Diagram of how the BBSome and primary cilium are proposed to affect leptin signaling. (**a**) Leptin receptors are observed at the trans-golgi network and in the plasma membrane. In response to leptin, increased phosphorylated STAT3 and *Pomc* transcript levels are observed along with a cessation of appetite. (**b**) The loss of BBS1 or BBS2 proteins results in a significant decrease in phosphorylated STAT3, and an accumulation of LepRb in vesicles at perinuclear regions. Thus feeding behaviour was proposed to require intraflagellar transport driven localization for efficient LepRb signaling.

and the BBSome are essential IFT components required for biogenesis of primary cilia. Defective leptin signaling at the primary cilium of POMC neurons may further disrupt satiety signaling and controlled food intake (Fig. 3).

Regulation of adipogenesis

The differentiation of adipocytes, which comprise the bulk of the fat-storage tissues, is subjected to the regulation of Wnt signaling. Most BBS proteins have been reported to be present in murine adipose tissues (99, 103–106). Forti et al. further noted a strong upregulation of several BBS transcripts concomitant with adipogenesis in mouse 3T3-F442A pre-adipocyte cell lines (106). Recent studies have begun to establish the relationship between the primary cilium, BBS proteins and adipogenesis.

In human pre-adipocyte cell cultures, Marion et al. observed a transient $9+2$ microtubule-based primary cilium in cells undergoing adipogenesis, which was absent in pre- and mature adipocyte populations. siRNAi-mediated knockdown of *BBS10* and *BBS12* expression in pre-adipocytes correlated with an inhibition of Wnt signaling characterized by decreased accumulation of β-catenin in the nucleus. An increased accumulation of PPARγ, a pro-adipogenesis factor was also observed in the nucleus, contributing to their differentiation into mature adipocytes (107). It was further noted that *BBS10*- and *BBS12*-deficient adipocytes derived from dermal fibroblasts of human patients accumulated higher levels of triglycerides, as well as had increased secretion of leptin. Together, these studies suggest that BBS proteins inhibit adipogenesis by regulating Wnt signaling at the primary cilium. BBS proteins or cilium functions may further influence the development of adipocytes to alter their capacities for, or the regulation of, triglyceride accumulation (Fig. 4).

A *C. elegans* model for lipid metabolism

Unlike mammals, an invertebrate animal *C. elegans* lacks adipocyte tissues and stores lipids in small droplets in intestine and hypodermis (108). Although lacking adipocytes, a recent study suggests that the molecular pathways that regulate lipid metabolism are conserved in *C. elegans* (109). It thus emerges as a promising genetic model to study lipid metabolism and obesity.

In a genome-wide screen for *C. elegans* mutants with increased fat accumulation, which was readily visualized by lipophilic dyes, Mak et al. uncovered a neuronal role for TUB-1 (TUBby-related) in lipid metabolism. Localized to the base of ciliated neurons, disruption of *tub-1* leads to an increase in lipid storage through affecting the development of sensory cilia. Interestingly, *tub-1* encodes a member of the conserved Tub-like protein family, and is the *C. elegans* ortholog of the mouse gene *Tub*, whose disruption led to late-onset obesity in the *Tubby* mouse through still elusive causes (11, 110–112). Initially identified as an obesity model, *Tubby* mice were later found to also display defects such as retinal degeneration and neurosensory hearing loss (11, 113), indicative of ciliary defects and their potential involvement in obesity.

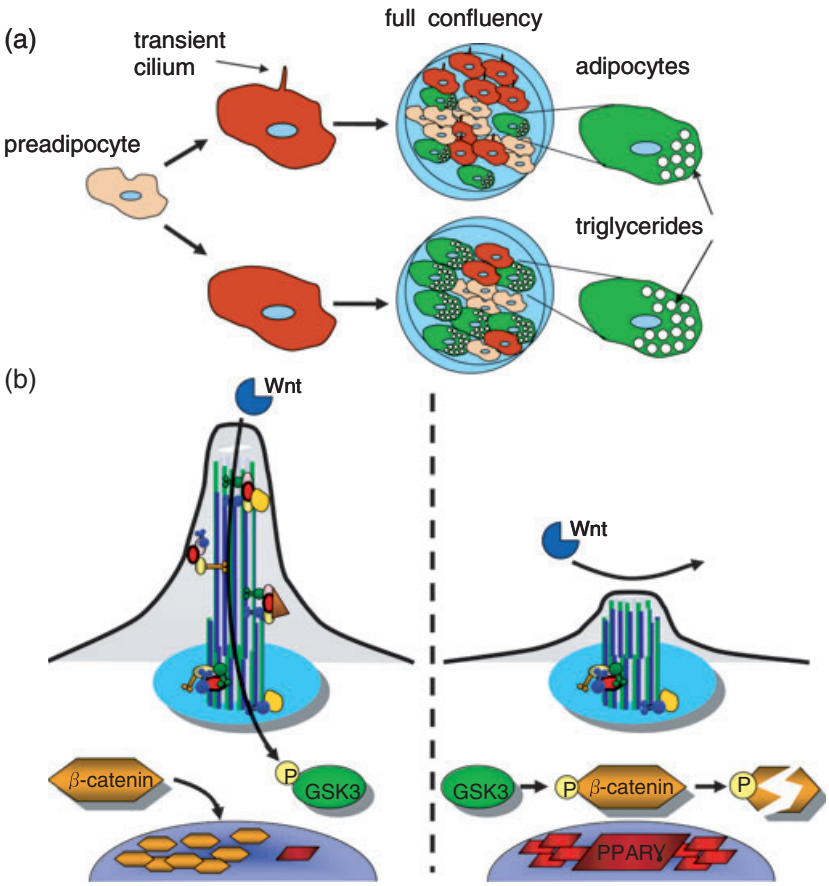

Fig. 4. A transient primary cilium inhibits adipogenesis. Diagram of the proposed involvement of the primary cilium in the regulation of adipogenesis. (**a**) A transient primary cilium present in white adipocyte tissue (WAT) undergoing adipogenesis was proposed to regulate Wnt signaling. In WAT cell cultures, the loss of *BBS10* or *BBS12* resulted in the loss of primary cilia and an increase in adipocyte populations at full confluency. Fibroblast cultures from human *BBS10* and *BBS12* patients at full confluency exhibited higher levels of triglyceride and leptin secretion. (**b**) The loss of the transient cilium in human white pre-adipocytes. Wnt signaling increases phosphorylation of GSK3 and β-catenin accumulation in the nucleus to inhibit adipogenesis. When the expression of BBS10 and BBS12 was reduced, a decreased GSK3 phosphorylation was observed concomitantly with a decreased accumulation of nuclear β-catenin and increased expression of pro-adipogenic peroxisome proliferator-activated receptor-γ.

Using *tub-1* mutants as a sensitized background for lipid accumulation, a genetic screen for phenotypic enhancers identified multiple alleles for 3-ketoacyl-CoA (*kat-1*)–a homolog of the human acetyl-CoA acetyltransferase (*ACAT1*). ACAT1 plays a role in mitochondrial fatty acid β-oxidation and is elevated in response to high-lipid intake. A slower pharyngeal pumping was observed when these genes were disrupted, excluding the possibility that the excess fat accumulation was caused by hyperphagy. Such a synergistic relationship between TUB-1 and KAT-1 thus supports a modulating role for cilia in lipid metabolism.

Mak et al. further identified an additional 41 synergistic loci that enhanced lipid accumulation in a *kat-1* mutant background. Intriguingly, amongst the most striking enhancers was a nonsense allele of *bbs-1*. Consistent with the neuronally restricted ciliary localization of *C. elegans* BBS proteins, BBS-1 was required in a specific subset of sensory neurons to prevent excessive lipid accumulation. Although the exact physiological relationship between BBS-1, KAT-1 and TUB-1 remains to be investigated, cilia also clearly partake in lipid homeostasis in invertebrates. A simple organism with fast generation time and amiable to large-scale genetic analyses, *C. elegans* is a promising animal model to reveal novel genetic components of lipid homeostasis and their contribution to obesity.

A potential contribution of ciliary genes in common obesity

Animal studies on molecular components that affect energy homeostasis were instrumental in the identification of genetic factors underlying monogenic forms of human obesity. For example, leptin (*LEP*), leptin receptor (*LEPR*), *POMC* and *MC4R* were first uncovered through obesity mouse models (19, 114), and later found to have causative associations with rare forms of monogenic obesity in humans (115–117). Heterozygous *MC4R* mutations in particular were associated with dominantly inherited obesity in two independent cohorts (118, 119), and with obesity in multiple ethnic groups (120–123), making it the most common cause for monogenic forms of obesity in human populations.

In contrast, although candidate gene studies in common obese populations also revealed an association with *LEP*, *LEPR*, *MC4R* and *POMC*, these

were the modest associations that failed genome-wide association significance tests (124–128). These studies suggest heterogeneous contributions in the development of common obesity.

Studies on human obesity disorders such as *BBS*, and animal studies in ciliary development revealed an intriguing link between ciliary dysfunction and the development of obesity. An unexpectedly large number of proteins comprise, and localize to this organelle; how most of these proteins regulate the assembly, maintenance and function of the cilium remains unknown (129). It is tantalizing to propose that the numerous ciliary components may exert small contributing effects that collectively affect the development of common forms of obesity.

Indeed, independent human genetics studies have shown that obligate, heterozygous BBS carriers were predisposed to obesity (130–133), and four intronic single nucleotide polymorphisms (SNPs) in BBS genes associated with common obesity in two large French-Caucasian populations were recently identified (134). Moreover, two recent genome-wide association studies identified several SNPs displaying ∼1% association with increased BMI (135, 136). Residing in an intronic region of *FTO*, FaT mass and obesity associated, a gene with unknown function, these variants may also affect a nearby gene *FTM*/*RPGRP1L*, as the expression of both *FTO* and *FTM/RPGRIP1L* was decreased in adipose tissues of various obese mice models. One of the associated SNPs was proposed to affect the expression of both genes through a shared Cutl-like 1 transcription factor-binding site (137). Localized at the basal body of cilia, *RPGRIP1L* has been implicated in Shh signaling in the mouse, and also causatively associated with two ciliopathies, the Meckel Gruber and Joubert syndromes (138–141).

Further examination of genetic components that affect ciliary development and function in regulation of satiety and lipid homeostasis in animal studies may reveal promising candidate loci affecting energy homeostasis and obesity in humans.

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