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The cilium: a cellular antenna with an influence on obesity risk

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Abstract

Primary cilia are organelles that are present on many different cell types, either transiently or permanently. They play a crucial role in receiving signals from the environment and passing these signals to other parts of the cell. In that way, they are involved in diverse processes such as adipocyte differentiation and olfactory sensation. Mutations in genes coding for ciliary proteins often have pleiotropic effects and lead to clinical conditions, ciliopathies, with multiple symptoms. In this study, we reviewed observations from ciliopathies with obesity as one of the symptoms. It shows that variation in cilia-related genes is itself not a major cause of obesity in the population but may be a part of the multifactorial aetiology of this complex condition. Both common polymorphisms and rare deleterious variants may contribute to the obesity risk. Genotype–phenotype relationships have been noticed. Among the ciliary genes, obesity differs with regard to severity and age of onset, which may relate to the influence of each gene on the balance between pro- and anti-adipogenic processes. Analysis of the function and location of the proteins encoded by these ciliary genes suggests that obesity is more linked to activities at the basal area of the cilium, including initiation of the intraflagellar transport, but less to the intraflagellar transport itself. Regarding the role of cilia, three possible mechanistic processes underlying obesity are described: adipogenesis, neuronal food intake regulation and food odour perception.

Key words: Obesity: Primary cilia: Adipocyte differentiation: Food odour sensation: Food intake regulation

Owing to its growing prevalence, obesity forms a global threat to public health and a burden to healthcare systems. Interventions by weight loss seem unable to bring this pandemic to a stop. Therefore, finding novel ways for treatment and prevention is a must, but depends on profound knowledge of the aetiology of obesity. Notably, the background of obesity is heterogeneous and complex, with involvement of genetic and environmental factors. Various physiological processes may be involved, including the response to environmental food cues, the hormonal regulation of hunger and satiety, the activity of the central reward system, whole-body energy expenditure and the storage capacity for fat in the adipose tissue. Each of these processes is related to the activity of a particular set of genes with some genes being involved in a broad spectrum of processes. Such genes are of special interest, because the pleiotropic effect of variation in those genes might explain a broader part of the aetiology of obesity. At the same time, those genes might be efficient targets for intervention.

Ciliary genes, which code for proteins required for the compartmentalised cilia biogenesis and function, represent such a group and have recently gained interest in the context of obesity. Cilia are specialised organelles extruding from eukaryotic cells (Fig. 1; more detailed information about the cilium structure and composition is available from the literature)[1–7], and are the product of a process that is referred to as ciliogenesis[8]. There are two types of cilia, motile and immotile/primary. Besides well-known motile cilia in the respiratory tract and the oviducts and immotile cilia of the inner ear and the nasal epithelium, almost every cell in the body carries a single primary cilium at a certain stage of its life cycle. Cilia regulate

Abbreviations: ADCY3, adenylate cyclase 3; ALMS1, Alström syndrome protein 1; ANKRD26, ankyrin repeat domain 26; ARL6, ADP-ribosylation factor-like 6; AURKA, aurora kinase A; BBS, Bardet–Biedl syndrome; BTC, basal transport complex; CCT, chaperonin containing TCP1; HDAC6, histone deacetylase 6; IFT, intraflagellar transport; IGF1-R, insulin-like growth factor 1 receptor; INPP5E, inositol polyphosphate-5-phosphatase E; KIF3A, kinesin family member 3A; NPHP, nephronophthisis; OR, olfactory receptor; PCM1, pericentriolar material 1; PDE6D, phosphodiesterase 6D; PI(4,5)P2, phosphatidylinositol (4,5)-diphosphate; POMC, pro-opiomelanocortin; PTHB1, parathyroid hormone responsive B1; RABL4, RAB-like 4; RPGRIP1L, retinitis pigmentosa GTPase regulator interacting protein 1-like; SDCCAG8, serologically defined colon cancer antigen 8; SHH, sonic hedgehog; TCTN1-3, tectonic family member 1–3; TTC21B, tetratricopeptide repeat domain 21B; WDPCP, WD repeat containing planar cell polarity effector; WDR10, WD repeat domain 10.

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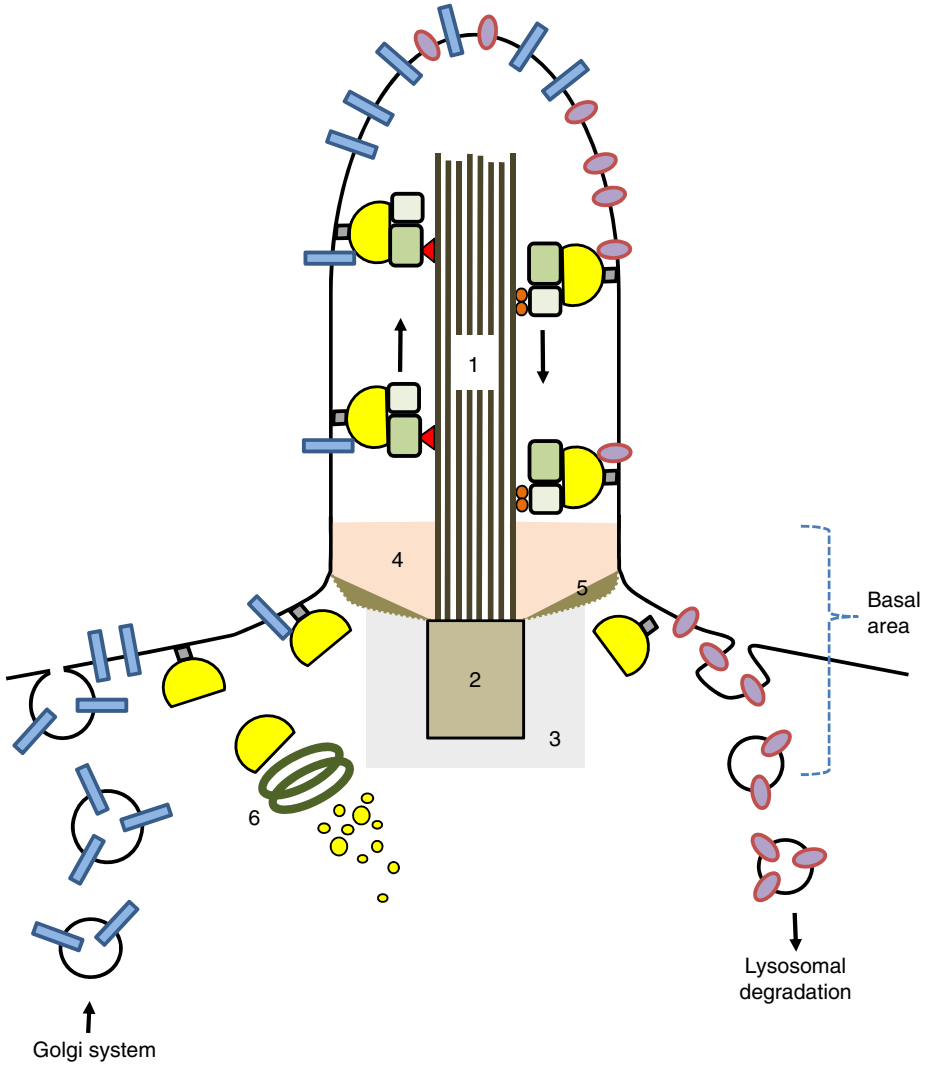

Fig. 1. Schematic representation of the cilium and the intraflagellar transport. 1, Axoneme; 2, basal body; 3, pericentriolar satellite; 4, transition zone; 5, transition fibre; 6, assembly complex; , membrane receptors; , structural Bardet–Biedl syndrome (BBS) proteins; , BBSome; , BBS3; , intraflagellar transport (IFT)-A; , IFT-B; , kinesin; , dynein.

the proper differentiation and migration of all kinds of cells in the body, are involved in signal transduction and allow cells to obtain environmental information as part of sensory systems[9–13]. Mutations in ciliary genes can cause monogenic disorders referred to as ciliopathies with pleiotropic consequences for the phenotype, which can include obesity. In this study, we focus on the obesity-linked subset of ciliary genes, review the clinical presentation of mutations, location of the ciliary proteins in the cell and their most likely function, and describe mechanisms by which they might contribute to the genetic background of obesity. In addition, we present our own experimental data of those genes in recent obesity and weight-loss studies.

Clinical presentation of obesity-linked ciliary genes

Because of the multiplicity of cilia functions, ciliopathy patients usually suffer from a range of symptoms and their condition is often classified as a syndrome. Examples of such conditions are the autosomal recessive Bardet–Biedl syndrome (BBS; MIM209900) and Alström syndrome (MIM203800). Among patients with different clinical entities due to a different mutated gene, symptoms may overlap[14]. For instance, BBS patients can present with retinitis pigmentosa, truncal obesity, renal dysfunction, polydactyly, behavioural dysfunction and hypogonadism as major symptoms, but also with diabetes, hepatic complaints and olfactory and/or auditory deficiencies. Alström syndrome is characterised by blindness, hearing loss, childhood obesity with hyperinsulinaemia and type 2 diabetes mellitus, but also by cardiomyopathy and by renal, pulmonary and/or hepatic dysfunction. Although mutations in different genes may cause similar symptoms, it is also possible that different mutations in the same ciliary gene lead to different clinical diagnoses. For instance, patients with mutations in the Meckel syndrome type 1 (*MKS1*) gene can present with BBS or Meckel–Gruber syndrome, whereas various mutations in *CEP290* may lead to BBS, Joubert or Meckel syndrome as well as Leber congenital amaurosis[15].

Some ciliopathy syndromes are genetically heterogeneous such as BBS, for which nineteen genes have already been reported[16]. For other ciliopathy syndromes, only a single gene has been identified, as is the case for Alström syndrome. Other single-gene, obesity-linked ciliopathies are a syndrome with mental retardation, truncal obesity, retinal dystrophy and micropenis (MIM610156)[17,18] and a syndrome with morbid obesity and spermatogenic failure (morbid obesity syndrome 1 or morbid obesity and spermatogenic failure syndrome, MIM615703). The latter is a condition with morbid obesity in humans, and in the mouse presents with obesity, hyperphagia and insulin resistance[19].

A patient is diagnosed with BBS when at least four of the following six major manifestations are scored: retinopathy, obesity, polydactyly, genital abnormalities, cognitive impairment and renal anomalies. It means that BBS patients do not necessarily have to be obese. In Table 1, we have listed various clinical conditions based on mutations in ciliary genes, and we have indicated for which of those genes obesity has actually been reported. It shows that for two of the BBS genes, *BBS11* and *BBS15*, obesity has not been reported as a symptom among patients. Interestingly, *Bbs11/Trim32*-knockout mice also do not become obese, although adult male mice have a 10 % increased body weight originating from the non-muscle mass[77]. When a mutated ciliary gene leads to Joubert syndrome, obesity is commonly not part of the symptoms. However, recently, a patient with Joubert syndrome and obesity has been reported with a homozygous missense mutation in the *ARL13B* gene, which affects ciliogenesis[21].

Besides clinical presentation, animal studies may also reveal new interesting genes in this cilia–obesity context. Recently, it was shown that knocking out the ankyrin repeat domain 26 (*Ankrd26*) gene in the mouse leads to defects of primary cilia in regions of the central nervous system, accompanied by hyperphagia, obesity and gigantism[78]. The human *ANKRD26* gene is situated in a locus for obesity on chromosome 10p with a maternal parent-of-origin effect[79]. However, mutations in this gene have not yet been described in connection to obesity in humans. Another gene of which mutations lead to symptoms including obesity in the mouse is *Tub*[80]. The protein of this gene is probably involved in the selective import of G protein-coupled receptors into cilia[81].

Functions of obesity-linked ciliary proteins and their closely interacting partners

The BBSome

The elucidation of the underlying genes in BBS led to the discovery of a special protein complex, the BBSome[82]. The function of the BBSome includes, but may not be limited to, the intraciliary (intraflagellar) trafficking of a diverse set of molecules (Fig. 1). This includes membrane-embedded receptors for signal transduction as well as structural components of the cilium. As such, the BBSome is important for the establishment, growth, turnover and functioning of cilia[83,84]. BBS1, BBS2, BBS4, BBS5, BBS7, BBS8 and BBS9 are part of the BBSome together with the 10 kDa BBSome interacting protein 1 (BBIP1), also referred to as BBIP10 or BBS18[44,85].

Chaperonin activity

Not all BBS genes code for components of the BBSome; three of the BBS genes code for chaperonin-like proteins: BBS3, BBS10 and BBS12. They share sequence homology with genes coding for members of the chaperonin containing TCP1 (CCT)/TCP1 ring complex (TRiC) family of chaperonins, which take part in ATP-dependent protein folding. The three BBSome proteins associate with six CCT-chaperonins, CCT1, CCT2, CCT3, CCT4, CCT5 and CCT8, to form a complex that is involved in the assembly of the BBSome[86]. The assembly starts with the interaction of BBS2 with BBS7 and BBS9, forming a core particle to which the other structural BBS proteins attach[87]. BBS18 appears to be important for enabling the incorporation of BBS4 into the BBSome[88]. It was observed that BBS2 is subject to ubiquitination. Therefore, turnover of BBS2 via the ubiquitin-proteasome pathway might be important for the regulation of BBSome quantity. BBS11/tripartite motif containing 32 (TRIM32), which has been shown to be an E3 ubiquitin ligase for actin and dysbindin, was suggested to be the processor of BBS2[87].

Intraflagellar transport

To enable intraflagellar transport (IFT), two other protein complexes are needed, IFT-A and IFT-B. The IFT-A particle is composed of IFT43 (c14orf179), IFT121 (WDR35), IFT122 (WD repeat domain 10; WDR10), IFT139, IFT140 (uncharacterized KIAA gene 0590; KIAA0590) and IFT144 (WDR19)[89], whereas the B-particle is composed of IFT20, IFT25, IFT27, IFT46, IFT52, IFT57, IFT72, IFT74, IFT80, IFT81, IFT88 and IFT172 (selective LIM-binding factor; SLB)[60]. Mediated by IFT144, the BBSome can interact with the IFT-A and IFT-B particles to form the basal transport complex (BTC), which moves along the central axoneme (Fig. 1). Membrane-embedded receptor proteins at the base of the cilium can dock to the BTC, and are then transported to the tip of the cilium. Although much of what we know about the function of these proteins has been collected from studies on *Chlamydomonas* and *Caenorhabditis elegans*, a similar model seems to be operative in mammalian cilia[84].

In a more advanced model, the IFT-B particle in connection with kinesins such as kinesin II and its component kinesin family member 3A (KIF3A)[90] drives the anterograde transport of proteins to the tip of the cilium, from which the IFT-A particle in connection with dyneins drives the retrograde transport[91]. Observations in the *Ift27*-knockout mouse indicate that IFT27, a component of the IFT-B particle, together with BBS17/leucine zipper transcription factor-like 1 (LZTFL1), facilitates the retrograde transport of BBS proteins and sonic hedgehog (SHH) receptors from the cilium[92]. *In vitro*, Liew *et al.*[93] found that IFT27 can bind to unloaded BBS3/ADP-ribosylation factor-like 6 (ARL6). BBS3/ARL6, after being loaded with GTP, is assumed to link the BBSome to the membrane[94,95]. What may happen is that, when the BTC reaches the top of the cilium, BBS3/ARL6-bound GTP is hydrolysed, perhaps by the action of the Rab-like GTPase IFT27, and as a consequence BBSomes are released from the membrane. Next, the release of IFT27 allows the activation of BBS3/ARL6 by GTP binding and attachment of

Table 1. Clinical syndromes due to mutations in ciliary genes

Disorders	MIM	Gene names	Obesity reported	References
Alström syndrome	203800	ALMS1	Yes	(20)
Joubert Syndrome 8	612291	ARL13B	Yes	(21)
Meckel Syndrome 9	614209	B9D1	No	(22)
Bardet–Biedl Syndrome 1	209900	BBS1	Yes	(15,23–25)
Bardet–Biedl Syndrome 2	615981	BBS2	Yes	(23,25,26)
Bardet–Biedl Syndrome 3	600151	BBS3/ARL6	Yes	(27)
Bardet–Biedl Syndrome 4	600374	BBS4	Yes	(23,25,28)
Bardet–Biedl Syndrome 5	615983	BBS5	Yes	(23,29)
Bardet–Biedl Syndrome 6	605231	BBS6/MKKS	Yes	(25,30)
Bardet–Biedl Syndrome 7	615984	BBS7	Yes	(23)
Bardet–Biedl Syndrome 8	608132	BBS8/TTC8	Yes	(23–25)
Bardet–Biedl Syndrome 9	615986	BBS9/PTHB1	Yes	(31)
Bardet–Biedl Syndrome 10	615987	BBS10	Yes	(15,23,25,32)
Bardet–Biedl Syndrome 11	615988	BBS11/TRIM32	No	(33)
Limb-girdle muscular dystrophy 2H	254110	Id.	No	(34)
Bardet–Biedl Syndrome 12	615989	BBS12	Yes	(23)
Bardet–Biedl Syndrome 13	615990	BBS13/MKS1	Yes	(15,35)
Meckel Syndrome 1	249000	Id.	No	(36)
Bardet–Biedl Syndrome 14	615991	BBS14/CEP290/NPHP6	Yes	(15)
Leber congenital amaurosis	611755	Id.	No	(37)
Joubert Syndrome 5	610188	Id.	No	(38)
Meckel Syndrome 4	611134	Id.	No	(39)
Bardet–Biedl Syndrome 15	615992	BBS15/WDPCP	No	(40)
Bardet–Biedl Syndrome 16	615993	BBS16/SDCCAG8	Yes	(41,42)
Senior–Loken Syndrome 7	613615	Id.	No	(23)
Bardet–Biedl Syndrome 17	615994	BBS17/LZTFL1	Yes	(43)
Bardet–Biedl Syndrome 18	613605	BBS18/BBIP1/BBIP10	Yes	(44)
Bardet–Biedl Syndrome 19	615996	BBS19/IFT27/RABL4	Yes	(45)
Meckel Syndrome 6	612284	CC2D2A	No	(46)
Joubert Syndrome 9	612285	Id.	No	(47)
COACH Syndrome	216360	Id.	No	(47)
Neuropathy, hereditary sensory, with spastic paraplegia	256840	CCT5	No	(48)
MOSPGF syndrome	615703	CEP19	Yes	(19)
Sensenbrenner syndrome (CED3)	614099	IFT43/C14ORF179	No	(49)
Short-rib thoracic dysplasia 2	611177	IFT80/WDR56	No	(50)
Meckel Syndrome-like	–	IFT88	No	(51)
Sessenbrenner syndrome (CED2)	613610	IFT121/WDR35	No	(49)
Sensenbrenner syndrome (CED1)	218330	IFT122/WDR10	No	(52)
Nephronophthisis 12	613820	IFT139/TTC21B	No	(53,54)
Mainzer–Saldino syndrome	266920	IFT140/KIAA0590	No	(55)
Short-rib thoracic dysplasia 9	266920	Id.	No	(56)
Nephronophthisis 13	614377	IFT144/WDR19	No	(57)
Retinitis pigmentosa 71	616394	IFT172	Yes	(58)
Growth hormone deficiency	–	Id.	Yes	(59)
Mainzer–Saldino syndrome	615630	Id.	Yes	(60)
MORM syndrome	610156	INPP5E	Yes	(17,18)
Joubert Syndrome 1	213300	Id.	No	(61)
Nephronophthisis 1	256100	NPHP1	No	(62)
Joubert Syndrome 4	609583	Id.	No	(63)
Senior–Loken Syndrome 1	266900	Id.	No	(64)
Nephronophthisis 4	606966	NPHP4	No	(65)
Senior–Loken Syndrome 4	606966	Id.	No	(65)
Joubert Syndrome 22	615665	PDE6D	No	(66)
Meckel Syndrome 5	611561	RPGRIP1L	No	(67)
COACH Syndrome	216360	Id.	No	(68)
Joubert Syndrome 7	611560	Id.	No	(67,69)
Joubert Syndrome 13	614173	TCTN1	No	(2)
Meckel Syndrome 8	613885	TCTN2	No	(70)
Joubert Syndrome 24	616654	Id.	No	(4)
Meckel Syndrome 3	610688	TMEM67	No	(71)
Joubert Syndrome 6	607361	Id.	No	(72)
COACH Syndrome	216360	Id.	No	(68)
Nephronophthisis 11	613550	Id.	No	(73)
Meckel Syndrome 2	603194	TMEM216	No	(74)
Joubert Syndrome 2	608091	Id.	No	(74,75)
Joubert Syndrome 14	614424	TMEM237	No	(76)

Id., idem; COACH, cerebellar vermis defect, oligophrenia, ataxia, coloboma, hepatic fibrosis; MOSPFG, morbid obesity and spermatogenic failure; MORM, mental retardation, truncal obesity, retinal dystrophy and micropenis. For expansions of gene names, see the text or the abbreviations list.

BBSomes to the membrane, in order to start the export of cargo proteins such as SHH receptors from the cilium[93]. Studies in the mouse and in zebrafish have suggested that another protein, clusterin associated protein 1 (CLUAP1), is also associated with IFT, possibly as part of the IFT-B particle, and may be involved in regulating the transport at the base and tip of the cilia[96,97]. The knockout mouse of this gene has a severe phenotype and dies at embryonic mid-gestation[96].

The basal area of the cilium

On the basal area of the cilium, three substructures can be distinguished: the basal body with transition fibres, the transition zone and the pericentriolar satellite. It is the area where many BBS proteins are active, and where the BBSome is assembled and the cargo proteins are uploaded or unloaded; two obesity-linked ciliary proteins, Alström syndrome protein 1 (ALMS1) and centrosomal protein 19 kDa (CEP19), are located at the basal body of the cilium and the subdistal centriolar appendage (transition fibre), respectively[19,98–100]. Absence of Alms1 in the mouse leads to changes in shape and orientation of cilia in hair cells[100]. Disruption of Alms1 in drosophila induces over-activation of the Notch signalling pathway, similar to after disruption of BBS1, BBS3 or BBS4, with the accumulation of Notch receptors in endosomes[101]. This observation suggests that ALMS1 and BBS proteins are involved in endosomal cycling and breakdown of signal transduction receptors occurring at the base of the cilium.

The transition zone is a region that forms the border between the cilium and the cell and prevents the free exchange of proteins between the cilium membrane and the plasma membrane[42]. Several proteins have been localised to this area, such as BBS13, BBS14, BBS15 and BBS16. In addition, particular complexes have been identified in this region such as the TCTN complex, which contains tectonic family member 1-3 (TCTN1-3), B9 protein domain 1 (B9D1), BBS13, BBS14, coiled-coil and C2 domain containing 2A (CC2D2A), transmembrane protein 67 (TMEM67) and TMEM216, and the HPNP complex composed of HPNP1, 4, 8 and retinitis pigmentosa GTPase regulator interacting protein 1-like (RPGRIP1L)[2,4,76,102]. It is suggested that they may function as gatekeepers for proteins that, being linked to the BBSome, are to be transported into and out of the cilium.

Investigations in embryos of knockout mice suggest that Rpgrip1l, which is located at the transition zone, in interaction with proteasome 26S subunit, non-ATPase 2 (Psmd2) regulates proteasome activity at the basal body of the cilium[103]. Although in humans no mutation in the *RPGRIP1L* gene has been reported in connection to obesity, the heterozygotes of the *Rpgrip1l*-knockout mouse are hyperphagic, have more fat mass and have a reduced suppression of food intake in response to leptin. In the hypothalamus of the heterozygous mice, the number of adenylate cyclase 3 (ADCY3)-positive cilia is decreased, with impaired localisation of the leptin receptor near the cilia, and reduced leptin signalling. A similar phenotype was observed in human fibroblasts with hypomorphic mutations in RPGRIP1L[104].

The BBSome may transiently interact with PCM-1, a major protein of the pericentriolar satellite. Through BBS1, the BBSome interacts with RAB interacting protein 8 (RABIN8), the RAS oncogene family member 8 (RAB8) nucleotide exchange factor. It stimulates GTP binding to RAB8, which in turn directs vesicles to the cilium for ciliary membrane elongation[82,105].

Inositol transduction and cyclic AMP signalling

The obesity-linked enzyme inositol polyphosphate-5-phosphatase E (INPP5E) has a function in inositol metabolism, hydrolysing phosphatidylinositol (4,5)-diphosphate (PI(4,5)P2) and phosphatidylinositol (3,4,5)-triphosphate (PI(3,4,5)P3), and as such is an important mediator of inositol signal transduction[106]. In addition, INPP5E activity regulates the phosphoinositide composition of the cilium membrane, which may have an influence on ciliary protein trafficking[107–109]. In the mouse, Inpp5e has been spotted in the axoneme[18]. Targeting of INPP5E into the cilium requires farnesylation and interaction with phosphodiesterase 6D[66,110]. Inactivation of INPP5E was shown to lead to cilium instability, which can be restored by blocking phosphoinositide 3-kinase (PI3K)[18]. Inactivation results in the accumulation of PI(4,5)P2 at the tip of the cilium and in depletion of phosphatidylinsositol 4-phosphate (PI4P). It attracts PI(4,5)P2-associated proteins such as Tubby-related protein (Tulp) and G protein-coupled receptor 161 (Gpr161), which induce changes in cyclic AMP (cAMP) production and Shh signalling[107,108].

Interaction of INPP5E with phosphodiesterase 6D indicates a link between inositol transduction and turnover of the second messenger cAMP. In fact, cilia contain various adenylate cyclases, of which type III (ADCY3) is relatively abundant. Links between the *ADCY3* gene and obesity have been reported both in humans and in mice. In a Swedish cohort, genetic association was found between variants of the *ADCY3* gene and men with obesity and type 2 diabetes[111]. In the Han population, genetic association with obesity was also observed[112]. *Adcy3*-knockout mice present with obesity, hyperphagia, low locomotor activity and leptin insensitivity[113]. Such mice also demonstrate anosmia towards IP3-and cAMP-generating odourants[114], which is not surprising, as olfaction depends on sensory cilia with a functional *ADCY3* gene.

Cilium stability

It has been observed that BBS18 is involved in microtubule stabilisation, for which its interaction with histone deacetylase 6 (HDAC6) is of importance[88,115]. HDAC6 deacetylates α-tubulin, and thereby de-stabilises the cilium[88,115]. It is activated by aurora kinase A (AURKA), which co-localises at the base of the cilium with neural precursor cell expressed, developmentally down-regulated 9 (NEDD9)/human enhancer of filamentation 1 (HEF1), a factor involved in the cilia-related cancer disorder von Hippel–Lindau syndrome[116]. In fact, the phosphorylation of HDAC6 depends on the interaction of AURKA with NEDD9[117]. Interestingly, INPP5E can also be activated by AURKA, which in turn down-regulates transcription of the AURKA gene[118]. Although activation of HDAC6 destabilises the cilium[88,115], activation of INPP5E has a stabilising effect[18]. In this regard,

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Table 2. Location and function of ciliary proteins

Genes	Cellular localisation and function	References
ALMS1*	Localises to the basal body of cilia	(99,120)
ARL13B*	Required for ciliogenesis	(21,121)
B9D1	Transition zone; basal body	(2,76,102)
BBS1*	Structural component of the BBSome, required for incorporation of BBS4	(87)
BBS2*	Structural component of the BBSome	(87)
BBS3/ARL6*	BBSome assembly, binding of the BBSome to the membrane	(93)
BBS4*	Final incorporated structural component of the BBSome, located at pericentriolar satellite (interacts with PCM1)	(87)
BBS5*	Structural component of the BBSome	(87)
BBS6/MKKS*	BBSome assembly	(87)
BBS7*	Structural component of the BBSome	(87)
BBS8/TTC8*	Structural component of the BBSome	(87)
BBS9/PTHB1*	Structural component of the BBSome	(87)
BBS10*	BBSome assembly	(87)
BBS11/TRIM32	Proposed E3 ubiquitin ligase for BBS2 in the assembly of the BBSome	(87)
BBS12*	BBSome assembly	(87)
BBS13/MKS1*	Part of the tectonic-like complex (transition zone complex); basal body	(2,4)
BBS14/CEP290/NPHP6*	Part of the tectonic-like complex (transition zone complex); centriolar satellite; basal body	(2,4)
BBS15/WDPCP	Present in the transition zone	(122)
BBS16/SDCCAG8*	Localises in the transition zone, present in the pericentriolar satellite (interacts with PCM1)	(23)
BBS17/LZTFL1*	Retrograde transport of SHH receptors	(123)
BBS18/BBIP1/BBIP10*	(de)Stabilisation of cilia by regulating de-acetylation of tubulin, BBS4-incorporation into the BBSome	(88,115)
BBS19/IFT27/RABL4*	Component of the IFT-B particle, plays a role in BBS3 activation during retrograde transport	(93)
CC2D2A	Transition zone; basal body	(4,102)
CEP19*	Transition fibre	(98)
IFT88	Component of the IFT-B particle	(60)
IFT172*	Component of the IFT-B particle	(124)
INPP5E*	Located in the axoneme, regulated by AURKA, cAMP and SHH regulation via inositol pathway	(18,107,118)
NPHP1	Transition zone	(4,102)
NPHP4	Transition zone	(2,4,102)
RPGRIP1L*	Transition zone; basal body	(2,103)
TCTN1	Transition zone, component of the tectonic-like complex	(2)
TCTN2	Transition zone, component of the tectonic-like complex; axoneme; basal body	(2,4)
TMEM67	Transition zone; axoneme	(2,102)
TMEM216	Transition zone; axoneme; basal body	(2,76)
TMEM237	Transition zone	(76)

SHH, sonic hedgehog; IFT, intraflagellar transport; AURKA, aurora kinase A; cAMP, cyclic AMP. For expansions of gene names, see the text or the abbreviations list.
* Mutation in the gene associated with obesity in human and/or mouse.

AURKA may play a key role in cilium turnover, and as such in the risk for obesity[88,107,115,118].

Pinpointing obesity to the basal area of the cilium

Mutations disrupting the function of the structural BBS proteins are supposed to hamper IFT, giving rise to obesity. As the IFT particles are associated with the BBSome, one would expect that mutations in the IFT proteins would also often lead to obesity. However, as can be seen in Table 1, this is only the case for BBS19/IFT27 and IFT172. The lack of obesity might be explained by a more severe phenotype of mutations, leading to prenatal or early postnatal death. Skeletal and renal abnormalities are common, in line with the function of primary cilia in those tissues[119]. It is also possible that with modern efficient sequencing methods, mutations in milder phenotypes of obesity are expected to be detected more often in the coming time. Alternatively, obesity is not so much a consequence of ciliary transport itself. As Table 2 shows, many of the obesity-linked ciliary proteins are located at the basal area of the cilium. In this region, the assembly of the BBSome takes place, its attachment to the membrane and its loading with the proper cargo protein. It is therefore tempting to speculate that obesity results from an altered initiation of the ciliary transport at the basal area of the cilium.

Genetic studies regarding obesity-linked ciliary genes

BBS is associated with truncal obesity. Compared with BMI-matched controls, BBS patients have a similar energy metabolism with higher visceral fat mass and higher leptin levels[125]. Apparently, these higher values are related to the underlying genetic defect[126]. BBS1 is for 80 % caused by the M390R missense mutation and patients show diet-responsive obesity[127]. For this mutation, a knock-in mouse has been generated[128]. The homozygous mice are obese, hyperphagic, have increased leptin levels and reduced locomotor activity. It proves that obesity is indeed a consequence of this gene mutation. Ultrastructural examination showed elongated cilia and swollen distal ends, but an intact axonemal structure[128]. Notably, phenotypic differences occur between the BBS subtypes. BBS10 patients have a significantly higher visceral fat mass than BBS1 patients[125]. Differences have also been observed with regard to the severity and age-of-onset of obesity. Comparing BBS2 patients and BBS4 patients, Carmi *et al.*[129] found that for BBS2 obesity was relatively mild,

whereas BBS4 was associated with early-onset morbid obesity. Further, genetic variants of BBS genes were found to be associated with different types of common obesity. In a study among French-Caucasian individuals[130], the association was found between *BBS2* (rs4784675) and common adult obesity, between *BBS4* (rs7178130) and *BBS6* (rs6108572) and early-onset childhood obesity, and between *BBS6* (rs221667) and adult severe obesity. Other studies did not show genetic associations. Variation in the *BBS6* gene among Danish obese subjects did not show significant association with common types of obesity[131]. Studying SNP for fourteen BBS genes in large cohorts of women did not show association with body weight or body fat, suggesting that common variation in BBS genes does not have a significant influence on body weight and fat[132]. This is in line with the outcome of a segregation analysis from 1995 by Reed *et al.*[133] in 207 sibling pairs, showing that BBS genes do not co-segregate with extreme obesity. Possibly, the penetrance of BBS gene mutations with regard to obesity is limited and/or the contribution to obesity risk depends more on rare alleles.

Previously, we sequenced thirty subjects with extreme obesity[134,135]. We checked for relatively rare variants (frequency < 0·01) with a predicted damaging impact on protein function in sixty-six genes that code for proteins, which are needed for proper cilia function (online Supplementary Table S1), including the genes mentioned in Tables 1 and 2. This resulted in the identification of twenty-four variants in seventeen subjects (Table 3). The *BBS5* N184S variant in subject 628 represents a serine substitution of an asparagine residue that is highly conserved within the two DM16 domains of the protein[29,136]; this has been reported before in two families with BBS5[136]. However, the two heterozygous patients of one of those families were also homozygous for the common M390R mutation in BBS1. Therefore, it was suggested that BBS5 interacts with BBS1 and that the N184S variant acts a modifier of the BBS phenotype. Besides these rare variants, we observed a non-synonymous SNP in *BBS10* (rs35676114, P539L) with a minor allele frequency of 0·07. For this SNP, we found association with extreme obesity in the examined cohort (χ^2, $P = 0{\cdot}0004$).

All subjects were heterozygous for the rare altered allele in line with the absence of syndromic symptoms. On the other hand, heterozygous variants may contribute to the phenotype as reported by McEwen *et al.*[37], who found reduction of smell perception in homozygotes and heterozygotes of the Cys998X mutation in *CEP290*. In this respect, the observed heterozygous variants might exert a small phenotypic effect. An accumulation of small effects from various rare alleles would fit with a multifactorial genetic background. Notably, subject 869 carried marked variations in four genes: *BBS1*, *IFT46*, *IFT88* and *ANKRD26*. Indeed, our observation shows the presence of a considerable number of rare alleles with a predicted

Table 3. Exome sequencing results of rare variants with a predicted impact on protein function in extremely obese subjects

					Functional prediction (reference)				Population
Genes	Person ID	rs-number	Nucleotide change	Amino acid change	SIFT†	PolyPhen2	Mutation taster	LRT prediction	Frequency
ADCY3‡	659	–	Gta/Ata	V860I	0·41	B	D	D	No
ANKRD26‡	869	139049098	caC/caG	H51Q	0·01	D	N	ND	0·0002§
	633	191015656	aCa/aTa	T181I	0·00	D	N	ND	0·001‖
BBS1‡	869	–	cGa/cAa	R440Q	0·01	D	ND	ND	No
BBS5‡	628	137853921	aAt/aGt	N184S	0·00	D	D	D	0·009‖
BBS8(TTC8)‡	698	140698625	Cgg/Tgg	R459W	0·00	D	D	N	0·001§
BBS9‡	588	–	tGt/tTt	C104F	ND	P	D	D	No
	692	59252892	aCt/aTt	T549I	ND	P	D	D	0·003§
BBS11(TRIM32)‡	593	117599771	caG/caC	Q186H	ND	P	D	D	0·006‖
BBS14(CEP290)‡	968	–	Gct/Act	A76T	0·52	D	N	D	0·001‖
CC2D2A	852/1003	144439937	Aag/Gag	K507E	0·29	D	N	D	0·009‖
	709	–	cGc/cAc	R1618H	0·02	D	D	D	0·00003§
CCT5	659	141675330	atC/atG	I362M	0·12	B	D	D	0·002‖
CCT7	646	200915592	Ggc/Agc	G377S	0·06	D	D	D	0·0006§
IFT121(WDR35)	852	138202017	Ggg/Agg	G93R	0·31	D	D	D	0·0002§
IFT144(WDR19)	588/612	201597047	Cgc/Tgc	R1223C	0·00	D	D	D	0·004‖
IFT46	869	145438119	Cct/Gct	P152A	0·03	D	D	D	0·007‖
IFT52	1314	148727335	Gag/Aag	E282K	0·17	P	D	D	0·001§
IFT80	588/612	375941259	Cga/Tga	R734*	ND	ND	D	U	0·0002§
IFT88	869	–	Gtt/Ttt	V704F	0·01	P	ND	D	No
NEDD9	41/646	34265420	cCa/cAa	P136Q	0·00	D	D	N	0·004‖
NPHP4	841	527701970	cCg/cTg	P301L	0·00	P	ND	D	0·0002§
TMEM67	698	202149403	aTg/aCg	M252T	0·00	B	D	D	0·0002§
	612	137853108	Aga/Tga	R208*	ND	ND	D	ND	0·0002§

SIFT, scale-invariant feature transform prediction method; PolyPhen2: LRT, likelihood ratio test; B, benign; P, possibly damaging; D, probably damaging; mutation taster: N, non-disease causing; D, disease causing; LTR: U, unknown; N, neutral; D, deleterious; ND, not determined; no, not present in these databases. For expansions of gene names, see the text or the abbreviations list.

* Stopcodon.

† SIFT: value < 0·05 is regarded as damaging.

‡ Mutation in the gene associated with obesity in human and/or mouse.

§ ExAc database non-Finish European frequencies.

‖ Genome of the Netherlands.

damaging impact on the proteins related to cilia function in extremely obese subjects. However, the actual involvement of this genetic variation in the risk for (extreme) obesity remains to be shown.

Another case (yet unreported data) concerns a male individual with early-onset severe obesity and anosmia. Exome sequencing revealed compound heterozygosity with two mutations in the *ADCY3* gene. One mutation is a frameshift (Gly423fs) retaining only one-third of the correct polypeptide sequence, whereas the other mutation is a deletion of a phenylalanine (Phe1118del). Using Provean prediction software (provean.jcvi.org), this mutation was classified as 'deleterious'. This finding in a male patient confirms the link between obesity and cilia function via the *ADCY3* gene as previously reported for *Adcy3*-knockout mice[113,114].

Although we did not find marked variation in the gene for RPGRIP1L in the thirty extremely obese subjects, from a genetic point of view this ciliary gene is particularly interesting, because its 5' end is only 100 bp from that of the gene for FTO, with overlapping promoters. FTO is one of the most studied genes in relation to the genetic risk for obesity. However, it has become clear that the FTO gene is part of a chromosomal segment, in which several genes are located that influence weight regulation[137,138].

Mechanistic role of cilia in obesity

Several mechanisms of how mutations in ciliary genes can contribute to increased body weight have been proposed[139–143]. In this study, we focus on three possible mechanisms: adipogenesis, central signalling of food intake and odour perception. Although these mechanisms are separately discussed, it should be kept in mind that mutations in cilia genes are pleiotropic and can increase the risk for obesity via more than one mechanism.

A primary cilium for adipogenesis

Adipogenesis occurs as a result of two opposing forces based on pro-adipogenic factors such as insulin-like growth factor 1 receptor (IGF1-R), CAATT/enhancer binding protein α-β (CEBP/A-B) and PPARγ and on anti-adipogenic signalling pathways such as SHH, wingless-type MMTV integration site regulatory gene/pathway (Wnt) and Notch. Ciliary proteins may influence either one or both of these forces, and have therefore been referred to as gatekeepers of adipocyte differentiation[144]. Marion *et al.*[144] showed that the reduced expression of the *BBS12* gene in mesenchymal stem cells down-regulated the anti-adipogenic pathways but promoted the pro-adipogenic factors. On the other hand, a decrease in Alms1, Ift88 or Kif3a expression inhibits cilium formation, as well as also adipocyte differentiation in mouse 3T3-L1 cells[145,146].

When human pre-adipocytes *in vitro* were induced to differentiate to mature white adipocytes, it was observed that a primary cilium appeared on the pre-adipocytes when cell cultures became confluent. Immunostaining showed in the cilium the presence of receptors involved in the SHH and Wnt signalling[147]. Similar observations were made in cultures of mouse 3T3-L1 pre-adipocytes, where a primary cilium together with α-tubulin acetylation was induced in growth-arrested confluent cells[146]. In those cells, a sensitised form of the IGF1-R, an important pro-adipogenic factor, was also detected in the cilium. Cilium formation could be inhibited by the suppression of Ift88 or the kinesin Kif3a[146].

Recently, the process of cilium formation was studied in more detail using *in vitro* differentiation of human mesenchymal stem cells into adipocytes. Within the first 2 d of differentiation, the primary cilium was observed to elongate together with increased trafficking of IGF1-Rβ into the cilium. This elongation process could be inhibited by insulin or by reduced IFT88 expression[148]. Similar information was obtained with *in vitro* differentiation of human adipose stem cells[149]. During the first few days after confluence, the primary cilium appeared and elongated, but thereafter it decreased in size to the stage where cells began to accumulate lipids. At that stage, the cilium completely disappeared. On day 3, approximately at the maximal length of the cilium, SHH signalling was reduced by 50 % as compared with undifferentiated cells. However, there is no definite proof that cilium length and SHH signalling are linked. Final disassembly of the cilium may involve the deacetylation of α-tubulin with microtubule destabilisation, by enzymes such as sirtuin 2 (SIRT2) and HDAC6[150]. Recently, the transient occurrence of the primary cilium during differentiation of human adipose stem cells was observed with disappearance of the cilium at the beginning of lipid accumulation[149].

Altogether, a picture emerges in which the primary cilium behaves like a sensory system that initially elongates and extends through the extracellular matrix (ECM) to monitor signals from the cellular environment[151]. This allows the cells to make a *go/no go* decision for differentiation. As the cells mature into lipid-loaded adipocytes, the ECM develops into a strong supportive layer and the cilium disappears.

Genetic variation or mutation in each of the ciliary genes may shift the balance between anti- and pro-adipogenesis differently, which may explain the variation in obesity phenotype between ciliopathy subtypes as mentioned before. In addition, the effect on the level of hyperplasia and hypertrophy may differ per gene. By studying *Bbs12*-knockout mice[144], it was observed that those mice had a higher number of small-sized to normal-sized adipocytes between hypertrophic cells than the wild-type mice. As small adipocytes are supposed to have a more healthy metabolic activity[152], this was seen as a possible explanation for the low risk of BBS12 patients to develop type 2 diabetes, whereas in Alström syndrome early-onset type 2 diabetes is common[20,153]. Knockdown of Alms1 in murine 3T3-L1 pre-adipocytes reduced pre-adipocyte differentiation by 2-fold[145], suggesting that obesity in Alström patients is accompanied mainly by hypertrophy.

Assuming that cilia monitor signals from the environment, it would be interesting to know how the genes mentioned here respond to changes in energy availability, but not much data have been reported. For eleven patients with obesity and type 2 diabetes, who underwent bariatric surgery, microarray analysis of blood cell RNA was performed before and 6 months after the surgery[154]. IFT121/WDR35 was among the seven genes, of

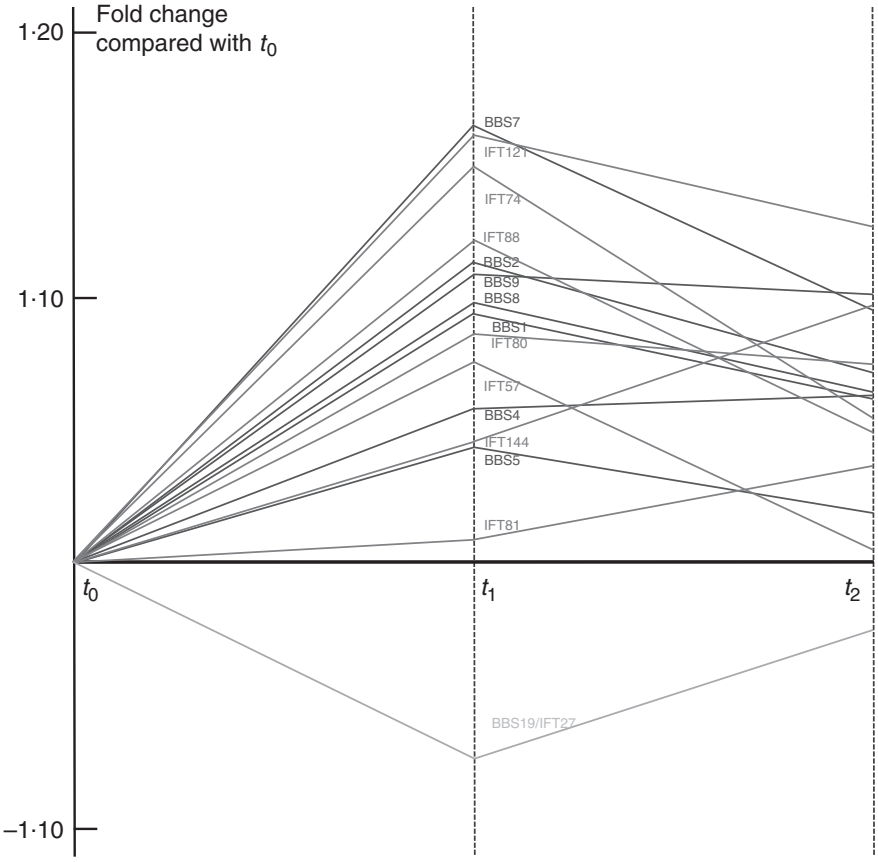

Fig. 2. Changes in expressions of genes coding for proteins of the basal transport complex (BTC) during energy restriction (t_0–t_1) and balanced energy intake (t_1–t_2). Expression changes are indicated as average (n 53) fold changes at t_1 and t_2 compared with t_0. ——, BBSome components; ——, components of the intraflagellar transport (IFT) particles; ——, changes in gene expression of BBS19/IFT27.

which the change in gene expression was strongly correlated with change in body weight, fasting plasma glucose and glycosylated Hb content.

To obtain further insight, we analysed gene expression data of fifty-three obese subjects who had lost approximately 10 % of their body weight either rapidly in 5 weeks or more gradually in 12 weeks on an energy-restricted diet, as reported elsewhere[155]. Adipose tissue RNA levels were measured by Affimetrix microarrays at the start of energy restriction (t_0), at the end of energy restriction (t_1, 5 or 12 weeks) and after subsequently having been on a weight-stable diet for 4 weeks (t_2). Fig. 2 shows the relative expression levels over time of fifteen genes coding for components of the BTC. In all, fourteen of the fifteen BTC genes were up-regulated during energy restriction. For half of the genes, the measured up-regulation was significant ($P \leq 0{\cdot}01$). The average up-regulation was 8 %, with the genes for BBS2, BBS7 and BBS9, from which the BBSome assembly starts[87], showing the highest up-regulation of 11 % ($P < 0{\cdot}001$), 17 % ($P < 0{\cdot}001$) and 11 % ($P = 0{\cdot}002$), respectively. This suggests that energy restriction stimulates IFT. Remarkably, the gene for BBS19/IFT27 was down-regulated by 7 %. This would leave BBS3/ARL6 more available for binding GTP, which would promote retrograde transport with possible (functional) decline of the cilium[93]. Such BTC profile might shift the balance between pro- and anti-adipogenic processes towards adipogenesis. An increased adipogenic capacity of the adipose tissue after energy restriction has been observed[156].

The cilium in neuronal food intake regulation

The hypothalamic neurons of genetic mouse models for obesity (ob/ob, db/db) have shorter cilia compared with lean mice (C57BL/6)[157]. The same observation was made for diet-induced obese mice with leptin resistance after a 14-week, high-energy diet. When 36-h fasted C57BL/6 mice were compared with mice that were re-fed afterwards for 6 h, an increased frequency of short cilia was seen after the fast and a higher frequency of longer cilia after the re-feeding[157]. Cilia length in these genetic models could be corrected by leptin supplementation, suggesting that the cilium length is increased by leptin. Using N1 hypothalamic neuronal cells *in vitro*, it was confirmed that leptin does not promote the number of ciliated cells, but increases cilium length[158]. This process seemed to depend on destabilisation of F-actin.

Rahmouni *et al.*[159] developed knockout mice for Bbs2, Bbs4 and Bbs6, which all showed increased food intake, body mass and fat mass. Administration of leptin could not bring down food intake and body weight, showing that these $Bbs^{-/-}$ mice are leptin resistant. Gene expression measurements pointed to

a defect in the pro-opiomelanocortin (POMC) neurons of the hypothalamus. Indeed, disruption of the cilia on the POMC neurons of the hypothalamus led to the obesity phenotype in mice[160]. In an additional study, it was shown that the leptin receptor interacts with BBS1, and that down-regulation of BBS1 or BBS2 in ARPE-12 cells induces mislocalisation of the leptin receptor[161]. Altogether, this suggests that the leptin receptor is transported by the BBSome into the cilia of hypothalamic POMC neurons and that a defect in this system results in hyperphagia, obesity and leptin resistance. Studying a conditional Ift88 deletion mutant with absence of cilia in the hypothalamus, Berbari *et al.* showed that leptin resistance only occurred in adult mice after they had become obese[162]. This observation was confirmed in the *Bbs4*-knockout study. It was concluded that leptin resistance is a secondary effect of obesity and not the cause.

Hypothalamic neurons also function in signalling pathways other than leptin signalling related to energy intake and energy homoeostasis. Probably, the cilia are key players in those processes as well. Loktev and Jackson showed that the cilia of hypothalamic neurons contain receptors for the orexigenic neuropeptide Y[163]. Moreover, the melanin-concentrating hormone receptor 1 (Mchr1), which is involved in food intake regulation, is normally present in the primary cilium. According to Berbari *et al.*[164], Mchr1 is not properly taken up in the cilia of *Bbs2*- and *Bbs4*-knockout mice, causing over-activation of the orexigenic melanin-concentrating hormone (MCH)-signalling pathway. In humans, association studies between the *MCHR1* gene and parameters of obesity are conflicting, which may be because of epigenetic effects[165].

Studying the obese/hyperphagic *Ankrd26*-knockout mouse, Acs *et al.*[78] recently demonstrated absence of Adcy3-containing primary cilia in the paraventricular nucleus, a part of the hypothalamus involved in food intake regulation. Similarly, disrupting the function of the *Alms1* gene leads to a large decrease of ciliated neurons in the hypothalamus as determined by the absence of Adcy3, Mchr1 and somatostatin receptor 3 (Sstr3)[166].

In summary, the findings indicate that cilia on hypothalamic neurons, particularly on the POMC neurons, contain the receptors for hormones that regulate food intake such as leptin, neuropeptide Y and MCH. Therefore, those cilia and their proper functioning are important for weight regulation. Either a reduction in the number of cilia or a change in length, which may be influenced by leptin, may induce hyperphagia and obesity.

Sensory cilia for odour perception

A reduction or ablation of odour perception referred to as hyposmia and anosmia, respectively, is a symptom of various ciliopathies. Hyposmia and anosmia have been shown to be cardinal and constant features in BBS[167]. A disturbance of the olfactory system has been shown in patients with BBS1, BBS3, BBS4, BBS5, BBS9, BBS10, BBS12 and BBS17, syndromes that are all associated with obesity (Table 1)[24,28,37,167].

Additional information on the link between ciliary genes, disturbed olfaction and weight regulation comes from animal studies. For several genes including *Bbs1*, *Bbs2*, *Bbs4, Bbs6* and *Bbs8*[24,159,168], genetic manipulation is accompanied by olfactory dysfunction, hyperphagia and/or increased weight. Knockout mice of *Bbs1* and *Bbs4* are runts of the litter at birth, but 10 % of them become obese at week 10[24]. Their odourant signalling is disturbed and the cilia of the olfactory epithelium show structural abnormalities with severe affection of the axoneme. In addition, the microtubular organisation of the dendrites is damaged[24]. *Bbs2*-knockout mice present with a deficit of olfaction and altered social behaviour[169]. Olfactory dysfunction presenting as partial or complete anosmia is also observed in the knockout of *Bbs6/Mkks* (McKusick–Kaufman syndrome)[170]. In the *Bbs8* knockout, a loss of olfactory cilia from the olfactory sensory neurons is observed as well as an altered pattern of axon targets[168]. Weight gain is slow at young age but eventually the adult mice develop obesity, which is especially pronounced in the females.

The IFT proteins have been far less studied than the BBS proteins for their possible involvement in olfaction and obesity. Knock-down of *Ift46* in the zebrafish led, among others, to disturbed ciliogenesis in the olfactory pits[171]. Similar observations have been made by Halbritter *et al.*[124] concerning a knock-down of *Ift172* with defective and shortened cilia in the zebrafish olfactory placode. A nonsense mutation in the gene for CLUAP1, supposed to be a part of the IFT-B particle, leads to absence of cilia from the olfactory pit of the zebrafish[171]. However, in none of those studies, a link with weight regulation was made. Mice homozygous for a hypomorphic mutation in the *Ift88* gene display polycystic kidney disease, underweight in litters and olfactory dysfunction, but do not develop obesity. However, this is suggested to be due to early death and health issues from multiple organ malfunction[172]. Ciliopathy in animals is not always accompanied by obesity, which is comparable with the situation in man. Moreover, direct evidence between cilia-related olfactory dysfunction and overweight/obesity is not yet available.

In more general sense, olfactory dysfunction may cause weight change, but does not always lead to overweight/obesity. Acquired hyposmia and anosmia may lead to all possible outcomes: either weight loss, weight gain or no change in weight[173]. Aschenbrenner *et al.*[174] found weight gain in 21 % of patients with acquired reduced sense of smell, whereas 11 % lost weight. Duration and severity of the affection and age may be of influence. Weight gain under olfactory deficiency is explained by a compensatory intake of nutrients, such as a higher amounts of sugar. Acquired reduction of smell in elderly women was observed to be associated with increased fat intake, suggesting risk for obesity[175]. In a small sample of persons with congenital anosmia (n 41), fifteen were overweight, which was significantly less than the expected twenty-six based on the frequency of overweight in the general population[174].

Although the relationship between the cilia-mediated olfactory system and overweight/obesity can be complex, a link on the molecular basis can be demonstrated. The olfactory sensory neurons form a dendritic knob from which sensory cilia protrude through the mucus layer of the nasal cavity. Those sensory cilia carry the olfactory receptors (OR), which are

transported into the cilia by the BTC[84,176]. As such, the cilium forms a link between the environment and the brain. Odourous compounds bind to the receptors, leading to depolarisation of the olfactory sensory cell[177]. This signal is transferred to the mitral cells, to which the sensory neuron is attached in the glomerulus of the olfactory bulb. From there, the signal is sent on to the hypothalamus and other regions in the brain. Experiments in the mouse have indicated that fluctuations in the level of secondary messengers, which in part are controlled by cyclases and phosphodiesterases, influence the way in which signals from odour-binding receptors are transferred[178]. Cilia-based olfactory defects may also relate to changes in the grey matter of the brain as shown by Braun *et al.*[167].

As the cilium is the intermediate in the signal from the environment to the brain, variation in the functioning of OR can be regarded as a mimetic for variation in the functioning of sensory cilia. In this regard, it is interesting to notice that genetic studies have shown a link between OR and food intake/obesity. Genetic association was demonstrated between OR7 genes and eating behaviour and adiposity[179]. Further, a copy number variant at chromosome 11q11 covering three OR genes, OR4P4, OR4S2 and OR4C6, was found to be associated with obesity[180]. In addition, we have reported associations between genetic variation in the OR14C36 gene and extreme obesity[135].

Besides the OR, the neuronal connections between olfactory sensory neurons and the mitral cells in the glomerulus are important for food intake regulation. Removing the olfactory bulb from Kv1·3$^{-/-}$ mice abolishes their resistance to diet-induced obesity[181]. It has been proposed that the development of the neuronal connections in the glomerulus depends on two opposing processes, axon guidance and repulsion, and involves both OR and clustered protocadherin genes[182]. We recently reported a relationship between genetic variation in the clustered protocadherin genes on chromosome 5q and extreme obesity[134]. Moreover, a genetic interaction between OR genes on chromosome 1q and the protocadherin-β genes has been observed in this extreme obesity cohort[135].

Notably, food odour can stimulate appetite, food-seeking behaviour and food ingestion, but strong or prolonged exposure to a food odour can have a satiating effect[183]. In this regard, stimulation of sensory cilia may induce increased appetite and food intake. On the other hand, diminished ciliary signalling of the OR neurons in ciliopathy syndromes might reduce satiating cues by food odours and promote food intake and obesity as well. This duality in the response to food odours complicates studies on the relationship between olfaction and obesity risk. Moreover, food perception and response is subject to the metabolic status[184]. Stafford & Whittle[185] showed that the preference and sensitivity to the odour of chocolate were different between obese and non-obese subjects. The property to vividly image flavours and aromas was found to be associated with BMI[186]. Despite this complex interaction between food odour and food intake, it is clear that cilia, particularly the sensory cilia, are essential for this process, and in this respect olfactory sensory cilia function may influence the risk for obesity.

Perspective on general obesity

As cilia are relevant for the proper development and performance of many cell types, organs and tissues, a mutation or, more in general, variation in a ciliary gene usually gives rise to pleiotropic effects. As a consequence, variation of a ciliary gene may contribute to the obesity risk from a broad spectrum of processes, including pre-adipocyte differentiation, hypothalamic regulation of food intake and olfactory perception and response. Despite the limited impact of variation in ciliary genes on obesity in general, the fact that those genes are players in various aetiological processes makes them an interesting target for intervention. The obesity phenotype in ciliopathies is a consequence of mutations that impair protein function. Therefore, overexpression of ciliary genes may provide a gene-therapeutic way to prevent obesity. Experiments with overexpression have been carried out to rescue the retinal phenotype of the Bbs1 knock-in mouse[128]. Overexpression of Bbs1 by AAV-Bbs1 injection into the retina improved symptoms of retinal degeneration. However, injection into the retina of WT mice led to outer retinal degeneration, demonstrating the potential risk of overexpression toxicity[187]. Perhaps a safer approach to explore the possibility of overexpression would be to aim for odour perception as part of the obesity risk via the olfactory sensory cells. The epithelium of the nose can be readily treated by gene therapy using non-invasive intranasal gene delivery[140]. As a demonstration of this application, adenovirus-mediated delivery of Ift88 to olfactory sensory nerves has led to restoration of the olfactory function in the Oak Ridge polycystic kidney disease (ORPK) mouse[51].

In a more optimal approach for obesity treatment, manipulation of ciliary gene expression should be performed by addressing various tissues to reduce simultaneously food craving, energy intake and fat storage in the adipose tissue. For this, drug therapy would be an attractive method. An example of potential drugs in this respect, although perhaps not very specific for mutations in ciliary genes, is the group of phosphodiesterase inhibitors, which can influence the level of second messengers. Not only can they have an effect on olfaction[178] but also on pre-adipocyte differentiation[188] and on leptin signalling in the hypothalamus[189]. Moreover, phosphodiesterase inhibitors exist as natural food components[190], although they have to be used with caution[191]. More knowledge on cilia and ciliary genes in relation to the risk of obesity should provide more specific ways for prevention and treatment in the future.

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There are no conflicts of interest.

Supplementary material

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