Transient ciliogenesis involving Bardet-Biedl syndrome proteins is a fundamental characteristic of adipogenic differentiation

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Bardet-Biedl syndrome (BBS) is an inherited ciliopathy generally associated with severe obesity, but the underlying mechanism remains hypothetical and is generally proposed to be of neuroendocrine origin. In this study, we show that while the proliferating preadipocytes or mature adipocytes are nonciliated in culture, a typical primary cilium is present in differentiating preadipocytes. This transient cilium carries receptors for Wnt and Hedgehog pathways, linking this organelle to previously described regulatory pathways of adipogenesis. We also show that the BBS10 and BBS12 proteins are located within the basal body of this primary cilium and inhibition of their expression impairs ciliogenesis, activates the glycogen synthase kinase 3 pathway, and induces peroxisome proliferator-activated receptor nuclear accumulation, hence favoring adipogenesis. Moreover, adipocytes derived from BBS patients’ dermal fibroblasts in culture exhibit higher propensity for fat accumulation when compared to controls. This strongly suggests that a peripheral primary dysfunction of adipogenesis participates to the pathogenesis of obesity in BBS.

Adipogenesis | primary cilium | ciliopathy | obesity

Origins of human obesity are complex and the study of inherited obesity syndromes is of great interest in identifying specific pathways that may be also implicated in more common forms. Bardet-Biedl syndrome (BBS), an autosomal recessive disorder with extensive nonallelic heterogeneity, is mainly defined by obesity, renal dysfunction, retinal degeneration, cognitive impairment, and polydactyly (1–3), and has been linked to a defect at the level of the primary cilium biology. The primary cilium is a microtubule-based organelle that protrudes from the surface of almost all human cells, acting as an antenna involved in extracellular signal transduction, implicating major biological pathways such as Wingless (Wnt) and Hedgehog (4, 5). Its importance has been recently highlighted by the growing number of inherited disorders related to ciliary defects (6, 7), illustrating the widespread tissular functions of this organelle. BBS is an emblematic ciliopathy with 12 genes identified to date (BBS1–BBS12), of which BBS1, BBS10, and BBS12 are cumulatively found mutated in more than 50% of the patients (1). Obesity is a cardinal feature of the disease, for which the ciliary pathogenesis remains to be clarified (8). The hypothesis of defects in the ciliated central nervous system neurons (9) that regulate fat storage has been explored and has gained recent support from studies of animal models (10–12). Moreover, as the adipocyte has been described to be a nonciliated cell (13) and is not referenced in the list of ciliated cells (http://www.bowserlab.org/primarycilium/cilialist.html), a direct implication of this cell in the pathogenesis of BBS-associated obesity has, so far, not been investigated.

Adipocytes are derived from mesenchymal precursor cells that, when they become committed to preadipocyte lineage, can either stay dormant or undergo terminal differentiation in mature adipocytes in a process described as adipogenesis (14). At this crossroad, several pathways antagonize each other: the antiadipogenic Wnt and Hh pathways are potent inhibitors of adipogenesis, whereas the peroxisome proliferator-activated receptor-γ (PPAR-γ) and CCAAT-enhancer-binding proteins (c/EBPα, β) are potent pro-adipogenic factors (15–17). Indeed, it is now clear that PPAR-γ is the master regulator of adipogenesis because it is able to stimulate normal levels of fat cell differentiation in cells lacking C/EBPα, whereas C/EBPβ has no ability to induce adipogenesis in absence of PPAR-γ (18). Wnt signaling maintains the preadipocytes in an undifferentiated state, and its inhibition is sufficient to cause spontaneous adipogenesis (19). Hh signaling also inhibits adipocyte differentiation, but unlike Wnt total repression, Hh signaling has been described to be only reduced during adipogenic differentiation with detectable levels present in mature adipocytes (20, 21). This down-regulation is not sufficient to trigger adipocyte differentiation, which makes Wnt signaling a more potent regulatory pathway of adipogenesis compared to Hh signaling.

Glycogen synthase kinase 3 (GSK3) is also a key regulator of adipogenesis (22) and is repressed by Wnt (19, 23). Indeed, when the Wnt pathway is active, GSK3 is inactivated through phosphorylation and is unable to phosphorylate β-catenin. This leads to the nuclear translocation of β-catenin, which represses differentiation. In contrast, in the absence of Wnt signaling, the unphosphorylated form of GSK3 is increased, which phosphorylates β-catenin targeting it for proteolytic degradation (24). This decrease in nuclear β-catenin is associated with the nuclear accumulation of PPAR-γ. Although it is well established that Wnt and Hh pathways are playing key regulatory roles in adipogenesis, the exact cellular localization of their corresponding receptors in preadipocytes has, to our knowledge, not been determined.

We approached the pathogenesis of obesity in BBS by investigating the role of cilia-related BBS proteins in the adipocyte biology. Indeed, an up-regulation of several BBS genes during the early phase of adipogenic differentiation in culture has been recently reported (25). In the present study we focused on 2 chaperonine-like BBS proteins that we recently identified: BBS10 (26) and BBS12 (27). This led us to discover the transient formation

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of a primary cilium that carries Wnt and Hh receptors, during preadipocyte differentiation. Inhibition of BBS10 and BBS12 expression impairs this ciliogenesis and activates proadipogenic pathways implicating GSK3 and PPARγ. Moreover, using adipocytes derived from BBS-patients’ dermal fibroblasts, we were able to show increased fat accumulation in the adipocytes and higher secreted leptin levels compared to control fibroblasts.

**Results**

**Cellular Localization of BBS10 and BBS12 Proteins.** As BBS10 and BBS12 proteins have not yet been characterized, rabbit polyclonal antibodies were raised that detect in Western blots single bands of the expected size (data not shown). We used them to study the intracellular localization of the 2 endogenous proteins. The kidney tubular epithelial cells represent a well-defined and recognized model for ciliated cells (28, 29), and moreover, kidney dysfunction is another cardinal feature in BBS patients (30, 31). We tested the localization of BBS10 and BBS12 in human primary renal proximal tubular epithelial cells (hRPTEC). When these cells were grown to confluence, their cilium was readily immunolabeled using anti-acetylated α-tubulin, with the basal body clearly seen at the base of the cilium (Fig. 1A). Immunodetection of BBS10 and BBS12 proteins showed that both proteins localized to the basal body of the primary cilium. Thus, these proteins appear to share the same localization as reported for BBS6 (32) that belongs to the same vertebrate-specific family of chaperonin-like proteins (27). This localization is thus more restricted than the localizations of the other BBS proteins belonging to the BBSome complex, which are also found in the cilium (33, 34). In mature adipocytes derived from human white preadipocyte (HWP) [SI Text and Fig. S1], BBS10 and BBS12 proteins were concentrated in 2 focal points colocalizing with the γ-tubulin-labeled centrioles, as shown in the merged pictures (Fig. 1B).

**Ciliated Differentiating Preadipocytes.** To analyze expression of BBS10 and BBS12 proteins during adipogenesis, we cultured the HWP to full confluence (D0) in preadipocyte growth medium, then switched to preadipocyte differentiation medium over 3 days, followed by a final change to adipocyte nutrition medium, leading to mature lipid accumulating adipocytes. Maximum expression levels of BBS10 and BBS12 proteins were detected in the first 2 days of adipogenic differentiation, followed by a net decrease as from day 4 to reach low basal expression levels (Fig. 2A). The glucose transporter4, GLUT4, was used as an adipogenic marker, expressed only in the mature adipocytes, to show that the BSS expression was progressively reduced with the proceeding of adipogenesis. This expression pattern appears thus similar to the previously described mRNA expression profile for the other BBS genes (25). The higher expression of ciliary proteins during adipogenesis prompted us to test for the presence of a cilium at 3 different stages: subconfluent dividing preadipocyte, confluent preadipocyte corresponding to maximum expression of BBS10 and BBS12, and mature adipocyte filled with lipid droplets (Fig. 2B). Interestingly, no cilium was detected in subconfluent preadipocytes and in mature adipocyte, but it was present in the confluent-differentiating preadipocyte. The same 3 cellular stages were examined by scanning electron microscopy, confirming the findings obtained by immunofluorescence labeling (Fig. 2C). Transmission electron microscopy revealed the typical ultrastructural architecture of the primary cilium in the differentiating preadipocyte with the 2 centrioles, 1 at the base of cilium in the basal body (Fig. 2D), and the presence of lipid droplets. At higher magnification, the axoneme is seen emerging from the cilium in the basal body, which could be counted to 9 + 2 doublets on a transverse section of the cilium (SI Text and Fig. S2C).

**Characterization of the Primary Cilium During Adipogenesis.** BBS1, one of the 2 most frequently mutated genes in patients, codes for a subunit of the recently described BBSome protein complex, the BBSome, and localizes within the primary cilium (33). We investigated whether BBS1 and the 2 chaperonin-like proteins studied herein share this same cellular localization in the differentiating preadipocyte. We costained the differentiating adipocyte for the cilium and for BBS1, BBS10, or BBS12. BBS1 was detected all along the adipocyte cilium (Fig. 3A) while BBS10 and BBS12 retained their cellular localization observed in unciliated fat cell, remaining associated to the cilium in the basal body of the primary cilium (Fig. 3B and C). In the renal epithelium, the primary cilium harbors receptors for pathways like Wnt and Hh (35), whose signaling is dependent on the inherent intraflagellar transport (36). We therefore tested if the differentiating preadipocyte primary cilium could be implicated in these pathways. We performed immunodetection of Wnt receptors using an antibody against a common epitope to the Fzrl receptors 1 to 10 (Fig. 3D). Specific Fzrl immunolabelling was found to localize in the cilium. In a similar way, we observed the presence on the cilium of Smoothened (Smo), a receptor involved in Hh signaling (Fig. 3E) as well as Patched1 (data not shown), suggesting the pivotal role of the cilium-associated signaling in the adipogetic program.

**BBS10 and BBS12 Affect Ciliogenesis and Adipogenic Pathways.** To investigate the implication of BBS10 and BBS12 proteins in the adipocyte transient ciliogenesis, we knocked down their expression using cellular Stealth RNAi delivery. Strong reduction of BBS10 and BBS12 protein contents were achieved in confluent D0 preadipocytes after treatment with the cognates RNAi (two different
RNAi for each gene gave similar results) (Fig. 4 A and B). Inhibition of BBS10 and BBS12 expression resulted in a significant reduction in the number of ciliated cells compared to control RNAi treated cells (Fig. 4 C and D). Because both BBS10 and BBS12 are chaperone-like proteins and are both localized at the centriole, we wondered whether one would need the other for its centriolar localization. We performed immunofluorescence for BBS12 in BBS10-deprived cells and for BBS10 in BBS12-deprived cells (Fig. 4 E and F) and no difference in BBS10 localization was observed after BBS12 deprivation or vice versa.

We then tested whether BBS10 and BBS12 knock down affect key regulators of adipogenesis as GSK3, β-catenin, and PPARγ based on an experimental procedure depicted in the scheme (SI Text and Fig. S3). Immunofluorescence detection of both the phosphorylated inactive and unphosphorylated active forms of GSK3β isoform in the preadipocytes was carried out. Upon BBS10 and BBS12 inactivation, the unphosphorylated active form of GSK3 was increased compared to control transfected cells (Fig. 5 A and B for ELISA quantification). Active GSK3 represses β-catenin nuclear accumulation (24). Cytoplasmic and nuclear fractions were therefore isolated from confluent preadipocytes after BBS10 and BBS12 inactivation and loaded on gel for immunodetection. Higher nuclear β-catenin protein content was detected in the control lane compared to the BBS10 and BBS12 knock-down lanes (Fig. 5 C, Right). Efficient separation between the nuclear and cytoplasmic fractions was tested by Western blot using Histone H3 as a control marker (SI Text; see Fig. S1 E and F). Indeed, histone H3 was detected only in the nuclear fraction, demonstrating good separation of the fractions. Concomitant to the reduction of nuclear β-catenin, inhibition of BBS10 and BBS12 expression induced a higher nuclear accumulation of PPARγ in differentiating preadipocytes compared to control cells (Fig. 5 D). This effect was confirmed by Western blot analysis (Fig. 5 E). The increased level of PPARγ is accompanied by its redistribution in the nuclei, appearing more homogenous than in cells that express normal levels of BBS10 and BBS12 proteins.

**Adipocyte-Derived Fibroblasts from Patients Have a Higher Propensity to Triglyceride Accumulation.** Dermal fibroblasts from BBS10 and BBS12 patients and healthy control were cultured in fibroblast growth medium. No difference in morphology was observed between the BBS10- and BBS12-deficient cells compared to the wild-type control cells (Fig. 6 A). At full confluence, the cells were differentiated into fat-accumulating cells as described in the SI Methods and Fig. S4. After 14 days, the fat vacuoles were visualized in the living cells (Fig. 6 B). The intracellular triglyceride (37) levels were measured in control and BBS-deficient cells and the corresponding fluorescence levels were plotted (Fig. 6 C). Significant increase in triglyceride content was observed in the BBS patients cells compared to the control cells. Concomitant to the increased level of triglycerides present in the cells with one BBS inactivated gene, there was a significantly higher leptin level secreted in the culture medium measured by ELISA (Fig. 6 D).

**Discussion**

The present study demonstrates that a primary cilium is present on the confluent differentiating preadipocyte (SI Text, see Fig. S2 A and B). This ciliogenesis is spontaneous when full cellular confluence is reached and doesn’t require serum withdrawal. In this respect, the HWP resembles the renal epithelial cells, which also exhibit spontaneous ciliogenesis. But at the difference of the renal monocilia, which is 9 + 0, the primary cilium carried by the human differentiating preadipocyte has a 9 + 2 (see SI Text and Fig. S2C) structure like the nonmotile kinocilium of hair cells in the cochlea (38, 39). Transient 9 + 2 cilia may act as developmental organelles at key points of cellular differentiation in the adipocyte and the kinocilium. The reported mouse models for BBS reproduce the human overweight condition (10). We verified that differentiating mouse preadipocytes (3T3-L1 cells) are also ciliated...
et al. Marion (see SI Text and Fig. S2D), indicating that this is a common feature in adipogenesis between these 2 species.

The transient ciliated status is accompanied by an increased expression of the 2 recently identified BBS genes BBS10 and BBS12, consistent with the previous findings that other BBS genes are also up-regulated during early adipogenesis (25). The chaperonine-like BBS10 and BBS12 show centriolar/basal body localization, whereas the other BBS protein, which forms part of the BBSome, have also been detected all along the cilium as exemplified by the BBS1 detection (see Fig. 3). One can therefore think that BBS10 and BBS12 are required, based on their predicted function of chaperonine, in assisting the formation of ciliary components and are not directly involved in intragellar transport. Both BBS10 and BBS12 appear essential for proper ciliogenesis during adipogenesis because their absence inhibits ciliogenesis, as shown in Fig. 4. However, they don’t require each other to localize to the centrioles, as no effect was detected on their respective localization following knockdown of the other one (Fig. 4 E and F). Alström syndrome, another syndromic obesity condition recently classified as a ciliopathy, is caused by mutations in the ALMS1 gene coding for another ciliary protein. ALMS1 was recently shown to be regulated during adipogenesis (40), further illustrating the prominent link between ciliary proteins and adipogenesis.

The primary cilium carries Wnt and Hh receptors, which are ancient signaling pathways and important well-recognized regulators of adipogenesis. Upon inhibition of BBS10 and BBS12 protein expression, the differentiating preadipocyte loses the cilium that carries Wnt and Hh receptors. This is likely the cause of the observed increase of the active (unphosphorylated) form of GSK3β, associated with a decrease in the nuclear content of β-catenin. GSK3β-mediated balance between β-catenin and PPARγ has been previously documented and the inactivation of

Fig. 4. Impaired cilium formation during adipogenesis upon BBS knock down. (A) Ninety percent confluent preadipocytes were transfected and cultured for 4 days in preadipocytes growth medium. Four days later, immunoblot analysis for BBS10 was done on control transfected RNAi duplexes (medium and low Negative Universal Control Stealth RNAi) (lanes 1 and 2, respectively) and on BBS10 RNAi knockdown confluent preadipocyte cells (BBS10 duplexes 1 and 2) (SI Methods, and lanes 3 and 4, respectively). Significant reduction in BBS10 protein level was obtained. Anti-β-Tubulin was used as loading control. (B) BBS12 was inactivated in a similar way and cell lysates analyzed by immunoblots (lanes 1 and 2 were loaded with cellular extracts from medium and low Negative Universal Control Stealth RNAi transfected cells and lanes 3 and 4 corresponded to those from cells transfected with BBS12 duplexes 2 and 3). A significant reduction in BBS12 level was also observed. (C) BBS10 or BBS12 silencing to prevent ciliogenesis. Four days after RNAi-transfection, cilia presence was assessed. Representative pictures of BBS10, BBS12, and control RNAi transfected cells are shown. Arrows indicate the primary cilium. (Scale bar, 40 μm.) (D) The percentage of ciliated cells: that is, (number of cilia/total number of nuclei) × 100% was calculated and plotted. n = 550 for each of 3 independent experiments. Significant reduction in ciliated cells was obtained (control compared to BBS10: P = 0.001 and control compared to BBS12: P = 0.003). (E) Immunolocalization of BBS10 in BBS10-deprived preadipocytes showed that centrifolar localization of BBS10 protein was unaffected in the absence of the BBS10. (Scale bar, 5 μm.) (F) In a similar way, BBS10 centriolar localization was also unaffected in BBS12-deprived preadipocytes. (Scale bar, 5 μm.)
Immunofluorescence for PPARγ was readily detected both by immunofluorescence and by Western blot at 48 h after induction of adipogenesis (15, 41, 42). The expression of PPARγ has been extensively studied in murine models, especially the 3T3-L1 preadipocytes, which require mitotic clonal expansion before they can start expressing the genes producing the adipocyte phenotype including PPARγ (43). A major difference exists between the widely studied 3T3-L1 preadipocyte model and the primary human preadipocyte used in the present study, as human preadipocytes can proceed through terminal differentiation without postconfluence mitosis (44), which may account for the earlier expression of PPARγ after adipogenic induction. To ascertain the adipogenic effect of BBS10 and BBS12 deprivation, we knocked down BBS10 and BBS12 in confluent preadipocytes and kept culturing them in the preadipocyte growth medium, a specially formulated medium to prevent adipogenesis, for 48 h before analyzing PPARγ expression (see SI Text and Fig. S2F). PPARγ was readily detected in the extract from the BBS10- and BBS12-deprived preadipocytes and remained almost undetectable in the control lysate. This proves that the absence of the cilium following BBS10 or BBS12 inactivation is sufficient to induce adipogenesis, probably by disrupting Wnt signaling, which could not be detected after the BBS knock down (data not shown).

**Fig. 6.** Increased fat content in adipocytes derived from BBS patients’ fibroblasts. (A) Representative pictures of human dermal fibroblasts with the indicated gene mutation cultured in FGM (SI Text, Methods) to full confluence before adipogenic induction. (Scale bar, 20 μm.) (B) Triglyceride accumulation after 14 days of culture in FGM was stained with Adipored Assay Reagent. Increased fluorescent-labeled cells were observable in the BBS10- and BBS12-mutated cells (Middle and Right, respectively) compared to control wild-type cells (Left). (Scale bar, 60 μm.) (C) The lipid-derived fluorescence was measured at 572 nm for each condition (CONT for the wild-type healthy fibroblasts, BBS10-, and BBS12-mutated fibroblasts) and was expressed as total measured fluorescence per cell. (n = 3) control compared to BBS10: P = 0.001 and control compared to BBS12: P < 0.001. (D) Secreted leptin was measured in the culture medium by ELISA and was expressed as the total absorbance at 450 nm per cell. In parallel with the increased intracellular triglyceride content, higher quantities of leptin was detected in the medium from the BBS mutated cells compared to the healthy control (n = 3) (control compared to BBS10: P = 0.05 and control compared to BBS12: P = 0.03).
Although stable gene knockdowns are useful, human fibroblasts from patients with characterized mutations often represent an invaluable tool to investigate the related diseases. Here, we were able to culture and reprogram dermal fibroblasts from two BBS patients. We then retrovirus transducing cells expressing PPARγ and GLUT4 (see SI Text, Methods, and Fig. S4). Interestingly, during the culture of these cells, no difference was observed in cell growth or in structural cellular aspects (data not shown), although it has been described that BBS6, the other chaperonine-like protein of the BBS family, blocked cytokinesis yielding abnormal poly nucleated cells (32). BBS6 protein may therefore possess a specific role in cell division, which both BBS10 and BBS12 don’t share. Based on the effect observed on PPARγ expression after specific BBS knockdown in the differentiating adipocytes, intracellular triglyceride content and secreted leptin levels were measured, and significant increase in intracellular triglyceride contents was observed in the BBS mutated cells compared to the control cells (see Fig. 6 B–D). It is well recognized that leptin secretion is directly correlated to adiposity. An increase in leptin secretion was measured in the culture medium from the BBS mutated cells compared to the control cells, reproducing the human-observed phenotype of high-circulating leptin concentrations.

The biological pathways leading to obesity in the BBS patients have been suggested to be related to central nervous system control of body weight. BBS proteins are required for proper ciliary localization in neurons of the Mchr1 receptor, involved in regulation of feeding behavior (12). Hypothalamic ciliated neurons may be involved in the leptin resistance recently described in BBS patients and obese BBS knockout mouse models (10, 45). BBS gene inactivation in these neurons has been shown to reduce Pomp expression, a gene activated by leptin via Stat3 (10). Nonetheless, this reduction cannot solely explain the increase in weight because the mice with specific inactivation of Stat3 and subsequent decreased Pomp expression exhibit a mild increase in total body weight and only a 2-fold increase in fat pad mass (46). The results presented in this article point out that the BBS-associated obesity may have a dual origin: the suggested central nervous system origin (10, 11) combined with a peripheral origin by increased adipogenesis via inhibition of Wnt signaling.

Lipodystrophy syndromes are to date the only syndromes related to primary abnormalities in adipogenesis. Obesity related to impaired adipogenesis has, so far, not been reported, and it is believed that body mass increase is not directly related to the adipocyte differentiation. Very recently however, Cao et al. (47) presented evidence for a lipid-mediated endocrine network where the adipose tissue itself uses lipokines to communicate with other organs and regulate systemic metabolic homeostasis. We therefore suggest that adipogenesis itself may participate to the pathogenesis of obesity. This hypothesis warrants further investigations in vivo. To address this question and to dissect the associated molecular parameters, conditional knockout mice will be used to target specific BBS inactivation in the adipose tissue, which will allow us to understand the role of enhanced adipogenesis in the BBS-induced obesity.

Materials and Methods

For details, please see SI Methods.

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Supporting Information

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SI Methods

Cell Culture. Primary hRPTEC (Catalog no.: CC-2553; Lonza) cultured in renal epithelial growth medium (Catalog no.: CC-3190), primary HWP (Catalog no.: C-12730; PromoCell bioscience alve) of abdominal origin were cultured according to manufacturer’s procedure. hRPTEC were grown to full confluence and left to differentiate for 8 days, yielding ciliogenesis. HWP were seeded in the Preadipocyte Growth Medium (Catalog no.: C-27410). Cells were grown to full confluence (D0) and then the medium was changed to the serum-free Preadipocyte Differentiation Medium (Catalog no.: C-27436) for 72 h. The medium was finally changed to Adipocyte Nutrition Medium (Catalog no.: C-27438) and the cells were fed every 3 days. 3T3-L1 preadipocytes (ATCC no. CL-173) were cultured in DMEM containing 10% FBS at 37 °C humidified 5% CO2 to full confluence.

Western Blots and Immunofluorescence Microscopy. Cells were lysed in a buffer containing 20-mM Tris-HCL (pH 7.5), 0.1% Triton X-100, 25-mM NaF, 12.5-mM NaPO4, 0.1-mM EDTA, 50-mM NaCl, 2-mM NaVO4 containing mixture of protease inhibitors Complete Mini EDTA free (Catalog no.: 11836 170001; Roche). For subcellular extraction of proteins from the cytosolic and nuclear fractions, the ProteoExtract Subcellular Proteome Extraction Kit was used (Catalog no.: 539790; Calbiochem). Protein concentration was determined using the Bradford reagent (Catalog no.: 500-0006; Bio-Rad). For Western-blotting, 100 μg of total protein lyzate were loaded per lane. Antibody binding was visualized using the Vectastain ABC-AmP Western Blotting Immunodetection kit (Catalog no.: AK-6001; Vector Laboratories). For immunofluorescence experiments, the cells were seeded on permanox 8-wells Lab-Tek II Chamber Slide (Catalog no.: 177445; NUNC). The cells were treated as indicated and were finally processed for protein detection after methanol fixation and permeabilized with 0.1% Triton x-100. The microscopy slides were mounted for detection with Vectashield Mounting Medium with DAPI (Catalog no.: H-1200; Vector Laboratories). Specific intracellular lipid staining was obtained using the Zeiss Axioplan2 microscope with either Zeiss lens 25X Plan Neofluar 0, 8 or with a 100X Plan Neofluar 1, 30 Oil Iris. All results shown are representative of at least 3 independent experiments.

Electron Microscopy. For scanning electron microscopy, samples were fixed by immersion in 2.5% glutaraldehyde in cadycad buffer (0.1 M at pH: 7.2) overnight at room temperature, washed in cadycad buffer for 30 min, and postfixed in 0.1 M cadycad buffer containing 1% weight by volume osmium tetroxide for 1 h at 4 °C. Cells were dehydrated through graded alcohol baths and dried with critical point-drying apparatus and mounted on aluminum stubs coated with palladium-gold using a cold sputter coater. Image acquisition was done with a Philips XL-20 Scanning Electron Microscope. For transmission electron microscopy, samples were fixed in Karnovsky fixative, postfixed with in 0.1 M cadycad buffer containing 1% weight-by-volume osmium tetroxide for 1 h at 4 °C. Samples were then dehydrated through graded alcohol and embedded in Epon 812 resin. Ultrathin sections of 70 nm were cut and contrasted with uranyl acetate and lead citrate. Pictures of the sections were made using a Philips Morgagni 268D transmission electron microscope.

Generation of Adipocytes BBS Knockdown. BBS10- and BBS12-specific knockdown were achieved using Stealth RNAi. The cells were cultured to 90% confluence and Lipofectamine 2000 (Catalog no.: 11668–019; Invitrogen) was used to deliver the siRNAs in the cells. The duplexes were individually transfected 3 times. Cells were analyzed 4 days later. The most efficient duplexes for BBS10 knockdown were 1 and 3 and duplexes 2 and 3 for BBS12 knockdown. Transfection efficiency was assessed with the Block-iT Alexa Fluor Red Fluorescent Oligo (Catalog no.:14750–100, Invitrogen). In parallel, cells were transfected with Stealth RNAi Negative Control duplexes (Catalog no.: 12935–100, Invitrogen). The appropriate negative control RNAi for each duplex was chosen based on the similarity in the percentage of GC between the control siRNA and the BBS siRNA.

Sequences of RNAi duplex (Invitrogen): For BBS10 were 5’ to 3’- duplex 1: CCG GCC UUA UAA UAA UUC UAC GCC UAA A; (Catalog no.: HSS128770) duplex 2: GGU CUC AAA GAU GUU GCC GCC UCU CAA A; (Catalog no.: HSS128771) duplex 3: CCA ACC UUU CUG GGA GAC CUC UUA A; (Catalog no.: HSS128772) For BBS12 were 5’ to 3’- duplex 1: GGA GGA UCA UUG GUC UUU CUC CAU U; (Catalog no.: HSS136498) duplex 2: ACG CUG ACA UGU UUG UCU UUG GAA A; (Catalog no.: HSS136499) duplex 3: CCA UUG ACA UGG UAA UCA CUG UAA A; (Catalog no.: HSS136500)

Negative Universal Control Stealth RNAi with Medium and Low GC content were used as controls (Catalog no.: 45–2001 and 45–2002, respectively)

Antibodies and Reagents. Primary antibodies used: rabbit polyclonal antibodies anti-BBS10 and anti-BBS12 directed against the apical domain of BBS10 and BBS12, respectively, expressed as fusion protein in bacteria. These 2 antibodies were produced at the rabbit polyclonal antibody facility of the Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC, Strasbourg, France). To verify our findings, commercially available antibodies were also used: BBS10 antibody was purchased from Proteintech Group, Inc., Catalog no.: 12421–2-AP, and BBS12 antibody was obtained from Abcam Catalog no.: ab67659; mouse monoclonal anti-β-Tubulin, mouse monoclonal anti-GSK3β (Phospho-Ser-9) and mouse monoclonal Anti-Histone H3 (Catalog no.: TUB-2A2, GSK-7G1-As, and H3-3E1–20, respectively, EUROMEDEX), rabbit polyclonal anti-Frizzled (H-300) (Catalog no.: sc-9169) used to detect frizzled proteins 1 to 10, goat polyclonal anti-BBS1 (Catalog no.: sc-49790), rabbit polyclonal anti-Smoothened (H-300) (Catalog no.: sc-13943), and rabbit-polyclonal anti-Patched (H-267) (Catalog no.: sc-9016) from Santa Cruz Biotechnology Inc., mouse monoclonal anti-γ-Tubulin, rabbit polyclonal Glucose Transporter GLUT4, and rabbit polyclonal GSK3β (Catalog no.: ab 11316–100, ab654 and ab 31366–100 respectively, Abcam), mouse monoclonal acetylated α-Tubulin (Catalog no.: 32–2700 Zymed laboratories, Invitrogen). Secondary antibodies used: biotinylated Anti-Rabbit IgG (H+L) Catalog no.: AK-6001, the
Biotinylated Anti-mouse IgG (H+L) Catalog no.: BA-2000, Vector Laboratories and DSB-X biotin donkey anti-goat IgG (H+L) Catalog no.: D20698, Molecular Probes for immunoblot analysis. For immunofluorescence experiments, antibodies used were: goat anti-mouse FITC (Catalog no.: 81–6511, Zymed laboratories), donkey anti-rabbit Texas Red (Catalog no.: sc6800, Abcam), and donkey anti-goat Texas Red (Catalog no.: sc-2783, Santa Cruz Inc.). Protein quantification of GSK3β was done with the ELISA STAR GSK-3β kit (Catalog no.: 17–471, Millipore).

Dermal Fibroblasts Derivation into Adipocytes. Human dermal fibroblasts were obtained in agreement with the patients who took part in Clinical Research Protocol (PHRC 2002) and informed consent was obtained as well as authorization from the Comite Consultatif De Protection des Personnes Dans La Recherche Biomedicale D’Alsace n°1 Strasbourg (File #/02/124). All medium supplements were purchased from Sigma Aldrich unless otherwise stated. Dermal fibroblasts were cultured in fibroblasts growth medium made of DMEM (Catalog no.: 31885; Gibco Invitrogen), 10% of FBS and Penicillin and Streptomycin (P/S) supplemented with 100-ng/ml Epidermal Growth Factor (Catalog no.: E9644), 1 μg/ml of Hydrocortisone (Catalog no.: H0888).

The cells were grown to full confluence before adipogenic induction was carried out by medium change to fibroblasts differentiating medium (FDM) for 3 days. FDM was composed of DMEM, P/S supplemented with 5g/L of D-Glucose, 0.5 μg/ml insulin (Catalog no.:I-5500), 8 μg/ml of d-Biotin (B-4639), 500 ng/ml of Dexamethasone (Catalog no.:D-4902), 50-μg/ml of IBMX (Catalog no.:I-58620), 10 ng/ml of L-Thyroxine (Catalog no.:T-1775), 5 μg/ml of Ciglitazone (Catalog no.: C-3974), and 5 μg/ml of Troglitazone (Catalog no.: T-2573). Following the 3 days of adipogenic induction, the cells were grown in a fibroblast-derived adipocyte medium (FDAM) made of DMEM, 5% FBS, P/S supplemented with 5g/L of D-Glucose and 1 μg/ml of insulin for 2 weeks before analysis.

Triglyceride and Leptin Measurements. The cells were seeded in 96-well plate and were cultured for 14 days in FDAM after adipogenic differentiation. On the fourteenth day, the cells were brought to room temperature and the medium was replaced with 200-μl PBS. AdipoRed reagent (5 μl) were added per well, the plates were mixed and incubated at room temperature for 10 min. After 10 min, the relative fluorescence was measured with an excitation at 485 nm and emission at 572 nm using a TECAN Polaris fluorimeter (Tecan Group Ltd.). Cell number was determined using a Neubauer hemocytometer. For leptin measurements, the cells were cultured in the FDAM in 10-cm Petri dishes with 10-mm medium. The medium was changed every 2 days until day 10 and was kept unchanged until day 14. On the day 14, the medium was harvested and stored at –80 °C until measurements were performed. Medium leptin concentration was determined using a Human Leptin ELISA kit (Catalog no.: RD191001100; BioVendor). For each condition, 100 μl of medium was used for the measurement according to the manufacturer’s procedure.

Statistical Analysis. Results are expressed as means ± SEM. To test if the averages between 2 samples were significantly different, we used a paired Student’s t test.
Fig. S1. In vitro differentiation of HWP. Preadipocytes and 6-day-old mature adipocytes were stained for the centrioles with γ-tubulin and the nuclei with Hoechst 33258 (A) and (B), respectively. Remains of lipid droplets were observed after fixation (asterisk). (C): Intracellular lipid in mature adipocytes was stained using the AdipoRed Assay Reagent in 2-week-old mature adipocytes. (Scale bars, 20 μm.) (D) Proteins after electrotransfer were stained using the Imperial Protein Stain (Catalog no. 24615, Pierce) to verify uniform loading before immunodetection of β-catenin: 1, control RNAi transfected; 2, BBS10 RNAi; 3, BBS12 RNAi. We loaded 30 μg of total protein per lane. (E) Another membrane loaded with the cytoplasmic and nuclear fractions stained before immunodetection of Histone H3 was performed in (F), showing good separation of the fractions, as the nuclear protein Histone H3 was only detected in the nuclear fraction and not in the cytoplasmic fraction: 1, control RNAi transfected; 2, BBS10 RNAi; 3, BBS12 RNAi.
Fig. S2. Kinetics of the ciliated status and cilia characteristics. (A) Differentiating preadipocytes were analyzed at the indicated time points. Cilia were detected as from D-1 to D4 using specific staining with acetylated alpha-tubulin. Nuclei were counterstained with Hoechst. (B) The total number of ciliated cells was counted at the indicated time points. Triplicates were done, and each time 550 nuclei were counterstained with Hoechst and the total number of cilia detected was counted. The percentage of ciliated cells was then determined and plotted. No ciliated cells were detected at D-4. Of the cells, 57% were ciliated at D-1 to reach a plateau at around 75% of ciliated cells between D0 and D4. At D8, only 16% of cells were ciliated. (C) Transverse section of a preadipocyte cilium showing the axonemes: 9 + 2 doublets can be seen. (D) Differentiating 3T3-L1 cells are also ciliated. After 48 h in differentiating medium, the cilium (acetylated alpha-Tubulin) was detected. (Scale bar, 10 μm.) (E), Cell lyzates of confluent preadipocyte 48 h after RNAi transfection and maintained in the preadipocyte growth medium were loaded for immunodetection of PPARγ. A specific band corresponding to PPARγ was detected in BBS10- and BBS12-deprived cell lyzates (lanes 2 and 3, respectively) whereas almost no PPARγ was detected in the control cell lyzate (lane 1). We loaded 30 μg of total protein per lane.
Fig. S3. Scheme depicting experimental procedure through time after BBS knock down. The scheme presents a time course for the human preadipocytes during adipogenesis, with the ciliated status indicated from D-1 to D+4. The preadipocytes were cultured to D-1 and all of the wells were transfected with either experimental BBS RNAi or control RNAi indicated on the scheme by BBS knockdown. One day later (D0), half of the plates were stopped and the effect of BBS knock down on GSK3 phosphorylated status and nuclear translocation of β-catenin was assessed. In the remaining plates, the medium was changed from preadipocyte growth medium to adipocyte differentiation medium. One day later (D+1), the differentiating adipocytes were analyzed for their PPARγ expression either by immunofluorescence (Fig. 5D) or by Western blot (Fig. 5E).
<table>
<thead>
<tr>
<th>Mutations</th>
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**Table of Clinical Pathologies**

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**Fig. S4.** Mutations and the associated clinical pathologies of the patients from which the dermal fibroblasts were reprogrammed in adipocytes and expression of adipogenic markers in the dermal fibroblast-derived adipocytes. (A) The BBS10 patient is a compound heterozygote having on one allele a frame shift leading to a stop codon and on the second allele a misense mutation causing an amino acid change from a cysteine to a tryptophan. The BBS12 patient is homozygous for a mutation in position 355, giving rise to a stop codon. The table presented summarizes the presence or absence of major characteristic pathologies associated with BBS. 1, present; 0, absent; *, not tested. (B) Dermal fibroblasts after 3 days of adipogenic induction in FDM were stained for PPARγ (Left), doubled-stained with PCM-1 (Middle), and the nuclei were counterstained with Hoechst (Right). All of the cells expressed PPARγ in their nuclei and PCM-1 was concentrated in a focal point. (C) Fourteen days after adipogenic induction, lipid droplets could be observed on living cells cultured in FDAM using the AdipoRed Assay Reagent. Immunofluorescence of PPARγ and its downstream target, Glut4, were detected in these cells (D). Nuclei were counterstained with Hoechst.