PHF2 is a JmjC family histone demethylase that removes the methyl group from H3K9me2 and works as a coactivator for several metabolism-related transcription factors. In this study, we examined the in vivo role of PHF2 in mice. We generated Phf2 floxed mice, systemic Phf2 null mice by crossing Phf2 floxed mice with CMV-Cre transgenic mice, and tamoxifen-inducible Phf2 knockout mice by crossing Phf2 floxed mice with CreERT2 transgenic mice. Systemic Phf2 null mice had partial neonatal death and growth retardation and exhibited less adipose tissue and reduced adipocyte numbers compared with control littermates. Tamoxifen-induced conditional knockout of PHF2 resulted in impaired adipogenesis in stromal vascular cells from the adipose tissue of tamoxifen-inducible Phf2 knockout mice as well as of Phf2 knocked-down 3T3-L1 cells. PHF2 interacts with CEBPA and demethylates H3K9me2 in the promoters of CEBPA-regulated adipogenic genes. These findings suggest that PHF2 histone demethylase potentiates adipogenesis through interaction with CEBPA in vivo. Taken together, PHF2 may be a novel therapeutic target in the treatment of obesity and the metabolic syndrome. Diabetes 62:1426–1434, 2013

The architecture of eukaryotic chromatin is dynamically modulated by posttranslational modifications of the histones, including acetylation, phosphorylation, ubiquitination, and methylation (1). Methylation states of histones are crucial for chromatin reorganization and regulation of gene transcription. For example, lysine (K) methylation at H3K9, H3K27, and H4K20 is associated with regions of transcriptionally silenced chromatin, whereas methylation at H3K4, H3K36, and H3K79 is associated with transcriptionally active regions. Such modifications are controlled by a balance between enzymes that catalyze the addition and removal of methyl groups. LSD1 and the Junonji C (JmJC) domain–containing proteins have been shown to possess such histone demethylase activities (2–4).

Plant homeodomain finger 2 (PHF2) is a newly characterized JmJC domain–containing protein identified as an interactant of nuclear receptors. PHF2 forms a complex with the AT-rich interactive domain 5B (ARID5B) and works as a coactivator for farnesoid X receptor (FXR) or hepatocyte nuclear factor 4 α (HNF4A). It is enzymatically inactive by itself but becomes an active H3K9me2 demethylase through protein kinase A (PKA)-mediated phosphorylation (5).

Although an increasing number of histone demethylases have been identified and their molecular functions progressively unraveled, the physiological functions of these demethylases remain largely unknown. Recently, LSD1 was reported to be required for embryogenesis (6), whereas JHDM2A is required for spermatogenesis (7) and obesity resistance (8) in vivo. In zebra fish, PHF8 and KDM7, which belong to the same subfamily of JmJC domain proteins as PHF2, regulate brain development (9,10). It has been suggested that PHF2 plays a role in induction of gluconeogenic genes by PKA signaling in hepatocytes (5) or rRNA expression in nucleoli (11) in vitro. However, in vivo analyses are required to explore the physiological role of PHF2. In this study, we generated PHF2 knockout mice and found that PHF2 plays a role in both neonatal growth and adipogenesis. These results imply that PHF2 demethylase function would be a novel translational target for human metabolic diseases.

RESEARCH DESIGN AND METHODS

Generation of Phf2 floxed mice by gene targeting. A bacterial artificial chromosome (BAC) DNA containing mouse Phf2 (BAC clone RP23-1141C14) was obtained from the BAC-PAC Resources Center. LoxP was inserted between exons 6 and 7 of Phf2 using the Escherichia coli-based BAC modification system (12). Modification cassettes were generated by PCR amplification of the PL42 vector with the following primers: 5'-TATATAGGACATCTTGACGAT-TCATGTCGTAATGATGGAAGCAGCTCAGTTGCAGCTCAGTCAGGAGCTCAGTGGTGCATACGATCTGTCAATTATCCGATCATATTCAATAACCC-3' and 5'-ACTCTGGACACTAGGTGACCCAGTGGCCTCTAATAGTTAATGAGCTCAGATCCATGGATGCCCCTGTTTCACTATCC-3'. The cassette vector was digested from the pNTR-lacZ-PGK-neo-lox vector and inserted into the pBSIIKS+ vector using the E. coli-based BAC recombination system. Modification cassettes and retrieval cassettes were generated by PCR amplification of the pBSIIKS+ vector with primers corresponding to each promoter. LoxP-P2-P6-Pk-Neo was digested from the pNTR-lacZ-PGK-neo-lox vector and inserted into the pMC1DTpA vector that contained LacZ-PGK-Neo and inserted into the pBSIIKS+ vector using the E. coli-based BAC recombination system.

Animal Care and Use Committee of The University of Tokyo.
Adipose tissue collection. Mice were killed by cervical dislocation. Epididymal, gonadal, subcutaneous, and mesenteric white adipose tissues (WATs) were carefully separated from testis, uterus, skin, and intestines, respectively. Wet weights of these WATs were measured.

Glucose and insulin tolerance tests. Blood was removed 2 h before glucose (2 g/kg) or insulin (1 units/kg) was administered by intraperitoneal injection. Blood samples were collected from the tail vein at various times after the glucose load, as indicated. Plasma glucose was immediately determined on an Accu-Check Aviva (Roche).

Cell culture. 3T3-L1 mouse fibroblasts were maintained and differentiated into adipocytes as previously described (15). Mouse primary embryonic fibroblasts were isolated on embryonic day 13.5 and cultured in Dulbecco’s modified Eagle’s medium with 10% FBS.

Western blot. Protein levels were determined by blotting with anti-FLAG antibody (Sigma-Aldrich), anti-CEBPA antibody (Santa Cruz Biotechnology), and anti-PHP2 antibody (5). ECL Plus (Amersham) was used for detection of the immunoreactive bands.

mRNA analysis. Total RNA was isolated with TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. First-strand cDNA was synthesized from total RNA using PrimerScript RT Master Mix (Takara) and subjected to real-time PCR using KAPA SYBR Fast qPCR Kits (Kapa Biosystems) with Thermal Cycler Dice (Takara) according to the manufacturers’ instructions. The following primers were used: mouse Phf2 5’-ATCATCTGGATGATGATTCT-3’ and 5’-TCTGAGTGATGATGATTCT-3’; mouse Cebpa 5’-GAGGAGGACGAGGAGAGG-3’ and 5’-AAAGATGG-3’; mouse Fabp4 5’-AGAACAGCAGCAAAATCAAGG-3’ and 5’-CTGGCCGGATGAGATAGAAGA-3’. First-strand cDNAs were synthesized using SuperScript II reverse transcriptase (Invitrogen). mRNA expression values were normalized to the genomic DNA concentration and using the spike RNA instructions.

Microarray analysis. These procedures were conducted according to the Perceivellme method (17). Briefly, mRNA expression values were normalized to the cell numbers in each sample by adding external spike mRNAs to them in proportion to the genomic DNA concentration and using the spike RNA quantity data as a dose-response standard curve for each sample. Total RNAs were purified from epididymal adipose tissue using an RNeasy Mini kit (Qiagen). First-strand cDNAs were synthesized using SuperScript II reverse transcriptase (Invitrogen). After second-strand synthesis, the double-stranded cDNAs were purified using a GeneChip (Affymetrix) Sample Cleanup Module and labeled by in vitro transcription using a BioArray High Yield RNA Transcript Labeling Kit (Enzo Life Sciences). The labeled cRNA was then purified using a GeneChip Sample Cleanup Module. Purified cRNA were hybridized with GeneChip Mouse Genome 430 2.0 Array. Washing and staining were performed in a GeneChip Fluidics Station using the appropriate antibody amplification, washing, and staining protocols. The phycoerythrin-stained arrays were scanned as digital image files, which were analyzed with GeneChip Operating Software. The expression data were converted to copy numbers of mRNA per cell by the Perceivellme method and analyzed using Perceivellme software.

Morphometric analysis. Paraffin sections of epididymal WAT were processed for hematoxylin-eosin staining and observed by light microscopy. Adipocyte areas were measured using OsteoMeasure.

Evaluation of adipocyte number. The number of adipocytes in epididymal WAT was estimated as previously described (18). Briefly, adipose tissue was minced and fixed at 37°C for 96 h in 1.88% osmium tetroxide. After washing twice with PBS, it was replaced with 8 mol/L urea at room temperature for 48 h. Urea-free particles were washed through a 180-μm net (Millipore), trapped on a 10-μm net (Millipore), and suspended using 0.01% Triton X-100 in PBS. Particles were counted with a counting chamber.

Oil red O staining. Cells were stained with oil red O, and the quantification was performed as previously described (19). Briefly, cells were fixed with 10% formalin and stained in a working solution of oil red O. Isopropyl alcohol was added to the stained culture dishes, and the extracted dye was monitored spectrophotometrically at 510 nm.

Statistical analysis. Data were analyzed by two-tailed Student t test, Pearson χ² test, log-rank test, or two-way repeated-measures ANOVA. For all graphs, data are presented as mean ± SEM. Statistical significance was accepted at P < 0.05.

RESULTS

Generation of Phf2 floxed mice. Figure 1A shows the strategy to generate the mutant Phf2 mutant allele. Successful insertion of LacZ-PGK-NeoR⁸ cassettes and loxP at sites flanking exon 6 of the Phf2 allele was confirmed by Southern blotting (Fig. 1B). Cre-mediated recombination excised exon 6 and PGK-NeoR⁸ from the mutant allele, resulting in the LacZ allele. P1, P2, and P3 primers were used to differentiate the wild-type and LacZ alleles (Fig. 1C). Flp-mediated recombination excised LacZ and PGK-NeoR⁸ from the mutant allele, resulting in a floxed allele. P4 and P5 primers were used to differentiate the wild-type and floxed alleles (Fig. 1D). Cre-mediated recombination excised exon 6 from the floxed allele, resulting in a null allele. Exon 6 corresponds to part of the JmJ domain of Phf2, and its deletion is expected to result in an alteration of the reading frame of the Phf2 transcript. As expected, the PHF2 protein was not detected in mouse embryonic fibroblasts of Phf2Z/Z (Fig. 1E).

Phf2Z/Z mice were characterized by partial neonatal death and postnatal growth retardation. Phf2Z/Z mice were born according to expected Mendelian ratios (Fig. 2A). However, <30% of Phf2Z/Z pups were alive when they reached 2 weeks of age (Fig. 2B). In fact, daily observation revealed that ~70% of Phf2Z/Z mice died within 3 days of birth (P = 3.0 × 10⁻⁴ compared with wild type) (Fig. 2C). Afterward, all remaining pups died by 10 days after birth, although there was little difference in body weight among newborn mice of different genotypes (Fig. 2D). Two weeks after birth, Phf2Z/Z mice grew in a manner similar to wild-type or heterozygous littersmates, but with significant differences in body weight (Fig. 2E). The extent of these weight reductions was not significantly different between males and females (two-way repeated-measures ANOVA). These data suggest that PHF2 is dispensable for the survival of embryos but is necessary for survival and growth in the neonatal period.

Reduced adipose tissue in Phf2Z/Z. Next, we surveyed the weight and appearance of each tissue present in male Phf2Z/Z mice at 5 weeks of age. As mentioned previously, Phf2Z/Z mice had decreased body weights at that time (Fig. 3A). Phf2Z/Z mice exhibited significantly shortened body lengths (Fig. 3A). Various tissues of Phf2Z/Z, including brown adipose tissue (BAT), appeared normal and weighed the same as in control littersmates. However, VAT and brain showed a different trend. The brain weights were significantly increased compared with wild-type littersmates, the potential significance of which will be discussed later (Fig. 3A). The weight of epididymal VAT was only 50% of that found in control littersmates, even when normalized by body weight. Weight reduction was also
observed in subcutaneous WAT to an extent similar to that in epididymal WAT (\( P = 0.06 \)) but to a lesser extent in mesenteric WAT (Fig. 3B). Additionally, female \( Phf2^{Z/Z} \) mice exhibited a similar reduced weight of gonadal WAT as observed in male mice (Fig. 3C). However, these weight reductions in adipose tissues seemed to be limited to a young age because there was no significant difference of WAT weights between \( Phf2^{Z/Z} \) and wild-type littermates when the mice reached 8 weeks of age (Supplementary Fig. 1). To elucidate whether the decreased weight of WAT was caused by a reduction of lipid droplets per cell or adipocyte number, we measured adipocyte size and number in epididymal WAT of \( Phf2^{Z/Z} \) and wild-type littermates. Evaluation of the mean adipocyte area in the section of WAT revealed that \( Phf2^{Z/Z} \) mice have smaller adipocytes than do control littermates (Fig. 3D). Furthermore, counting the number of adipocytes in WAT revealed that \( Phf2^{Z/Z} \) mice have fewer adipocytes in adipose tissue (Fig. 3E). These data suggest that the decreased weight of WAT in \( Phf2^{Z/Z} \) mice was caused by a reduction in both the size and the number of adipocytes. Because impaired adipogenesis often results in decreased size (20) and a decreased number of adipocytes, we used microarray analysis to compare the gene expression profiles in WAT between \( Phf2^{Z/Z} \) mice and control littermates. As a result, the expression of various genes associated with adipogenesis, such as \( Pparg \), \( Cebpa \), \( Fabp4 \), \( Adipoq \), \( LPL \), \( Plin2 \), and \( Cd36 \), tended to be decreased in \( Phf2^{Z/Z} \) mice (Supplementary Fig. 2). The expression levels of more than half of these genes were significantly reduced when confirmed by real-time quantitative PCR (qPCR) (Fig. 3F). These data suggest that decreased WAT weight in \( Phf2^{Z/Z} \) mice resulted, at least in part, from impaired adipogenesis in these mice. Finally, we measured insulin sensitivity in

FIG. 1. Strategic scheme for targeted disruption of mouse \( Phf2 \). A: Targeting strategy with positive/negative selection. Strategy of genomic Southern blotting in the screening for homologous recombinant embryonic stem cell clones is also included. E5, E6, and E7 represent exon 5, exon 6, and exon 7 of \( Phf2 \), respectively. B, H, E, and N represent BglII, HindIII, EcoRI, and NsiI cut sites, respectively. P1, P2, P3, P4, and P5 represent locations of primers used in C and D, respectively. \( LoxP \) sites; \( Frt \) sites. B: Southern blotting analysis of targeted embryonic stem cell clones. Restriction enzymes used for screening recombination events with probe A were BglII and HindIII. An 8.3-kb fragment in WT and a 5.3-kb fragment after homologous recombination were expected with probe B. Restriction enzymes used for screening recombination events with probe B were EcoRI and NsiI. A 9.0-kb fragment in WT and a 7.2-kb fragment after homologous recombination were expected with probe B. C: To detect the presence of the \( LacZ \) allele (\( Z \)) and the WT allele (+), primers P1, P2, and P3 were used. The PCR bands of the WT allele (242 bp) and the \( LacZ \) allele (495 bp) are indicated. D: To detect the presence of the floxed allele (\( fl \)) and the WT allele (+), primers P4 and P5 were used. The PCR bands of the WT allele (162 bp) and the floxed allele (245 bp) are indicated. E: Western blot analysis of PHF2 protein expression in \( Phf2^{Z/Z} \) mice. Extracts of mouse embryonic fibroblasts from WT or \( Phf2^{Z/Z} \) were immunoprecipitated and detected with anti-PHF2 antibody. WT, wild type.
mice because adipose tissue is well known to be involved in glucose metabolism. There was no significant change in the glucose or insulin tolerance tests (Supplementary Fig. 3), suggesting that reduced WAT weight did not affect insulin sensitivity in mice at 5 weeks of age.

PHF2 is necessary for normal adipogenesis. To elucidate the role of PHF2 in adipogenesis, we generated \( \text{Phf2}^{fl/fl} \); \( \text{Cre-ERT2} \) mice by crossing \( \text{Phf2}^{fl/ox} \) mice with \( \text{Cre-ERT2} \) mice. Stromal vascular cells (SVCs) were then obtained from \( \text{Phf2}^{fl/fl}; \text{Cre-ERT2} \) mice or control \( \text{Cre-ERT2} \) mice and treated with 4-hydroxytamoxifen (4-OHT) to induce Cre-mediated excision of \( \text{Phf2} \). As expected, treatment with 4-OHT efficiently reduced mRNA expression of \( \text{Phf2} \) in \( \text{Phf2}^{fl/fl}; \text{Cre-ERT2} \) but not in control \( \text{Cre-ERT2} \) mice (Fig. 4C). When SVCs were treated with a differentiation cocktail to induce their differentiation into adipocytes, differentiation was impaired in 4-OHT-treated SVCs from \( \text{Phf2}^{fl/ox}; \text{Cre-ERT2} \) mice as shown by oil red O staining (Fig. 4D and E) and expression of adipogenic marker genes (Fig. 4F).

PHF2 is recruited with CEBPA to the promoter regions of adipogenic genes. The results indicated that PHF2 promoted adipogenesis. Moreover, we show that PHF2 works as a coactivator for several transcription factors through H3K9me2 demethylation (5). Therefore, we hypothesized that PHF2 coactivates transcription factors that promote adipogenesis. Among such transcription factors, PPARG and CEBPA are the master regulators for adipogenesis (21). First, we assessed whether PHF2 could physically interact with PPARG or CEBPA. When transfected into HEK293 cells, FLAG-CEBPA (but not FLAG-PPARG) was coimmunoprecipitated with endogenous PHF2 (Fig. 5A). This was also confirmed by decreased expression of adipogenic marker genes in 4-OHT-treated SVCs from \( \text{Phf2}^{fl/ox}; \text{Cre-ERT2} \) mice (Fig. 4C). Next, we generated 3T3-L1 cell lines in which PHF2 was stably knocked down by retrovirus carrying \( \text{Phf2} \)-targeted short hairpin RNA. Infection of retrovirus carrying sh\( \text{Phf2} \) successfully decreased the expression of \( \text{Phf2} \) (Fig. 4F). In accordance with the results from SVCs, short hairpin RNA-mediated knockdown of \( \text{Phf2} \) resulted in impaired adipogenesis in the 3T3-L1 cell line as assessed by oil red O staining (Fig. 4D and E) and expression of adipogenic marker genes (Fig. 4F).

**FIG. 2.** Physiological features of systemic \( \text{Phf2} \) knockout mice. A: Genotypes of progeny of crosses between \( \text{Phf2}^{Z+} \) at birth. B: Genotypes of progeny of crosses between \( \text{Phf2}^{Z+} \) at 2 weeks of age. C: Survival rate of progeny of crosses between \( \text{Phf2}^{Z+} \). D: Growth curves of wild-type, heterozygous, and homozygous \( \text{Phf2} \) knockout mice until 10 days after birth. E: Growth curves of male mice and female mice of indicated genotypes between 2 and 10 weeks of age. ◻, wild-type mice (+/+); ■, heterozygous \( \text{Phf2} \) knockout mice (Z/+); ▲, homozygous \( \text{Phf2} \) knockout mice (Z/Z). *\( P < 0.05 \); **\( P < 0.01 \) compared with wild type.
FIG. 3. Assessment of adipose tissue of systemic Phf2 knockout mice. A: Ratio of body weight and nasoanal length or normalized tissue weight of male Phf2<sup>−/−</sup> to WT littermates at 5 weeks of age. Tissue weights were normalized to body weights (n = 6). B: Weights of epididymal (epi) WAT, subcutaneous (sub) WAT, and mesenteric (mes) WAT of male Phf2<sup>−/−</sup> KO mice and WT littermates at 5 weeks of age (n = 3). C: Weights of gonadal WAT of female Phf2<sup>−/−</sup> KO mice and WT littermates at 5 weeks of age (n = 4). D: Mean adipocyte areas of epididymal WAT from Phf2<sup>−/−</sup> KO mice and WT littermates (n = 5). High-magnification micrographs of WAT are shown. E: Adipocyte number in epididymal fat pads of Phf2<sup>−/−</sup> KO mice and WT littermates (n = 7). F: Real-time qPCR analysis of adipocyte marker genes and Phf2 of Phf2<sup>−/−</sup> KO mice and WT littermates (n = 3). *P < 0.05; **P < 0.01 compared with WT. KO, knockout; WT, wild type. (A high-quality color representation of this figure is available in the online issue.)
3T3-L1 adipocytes (Fig. 5C). These results indicate that PHF2 might work as a coactivator for CEBPA. PHF2 seemed to be necessary for recruitment of CEBPA to chromatin because the recruitment of CEBPA to CEBPRE was impaired in 3T3-L1 in which Phf2 had been knocked down (Fig. 5D). Moreover, modification of H3K9me2 in these CEBPRE was significantly increased in Phf2 knocked-down 3T3-L1 adipocytes (Fig. 5E). These data indicate that PHF2 can play
a role as a coactivator, positively regulating adipogenic gene expression with CEBPA through H3K9me2 demethylation near CEBPA-binding regions.

DISCUSSION
To investigate the role of PHF2 in vivo, we generated Phf2 knockout mice. Phf2 knockout mice showed partial neonatal death, growth retardation, and reduced body weight. Reduced body weight seems to be mainly related to growth retardation and reduced lean mass in the knockout mice rather than reduced WAT mass because the body weight of Phf2 knockout mice is still significantly lower than that of wild type at 8 weeks of age (Fig. 2E) when the weight of WAT is not different between Phf2 knockout and wild type (Supplementary Fig. 1). The reason for this phenotype is not clear. PHF2 has been reported to be highly expressed in the neural tube and dorsal root ganglia (22), and Phf2 represents a candidate gene for hereditary sensory neuropathy type I (HSN1) (23). In fact, the brain weights of Phf2 knockout mice were larger than wild-type littermates. Taken together, we speculate that Phf2 knockout mice may exhibit partial neonatal death attributable to defects in the central nervous system. Conditional deletion mutants using brain-specific Cre mice would clarify the precise roles of PHF2 in brain development.

On the other hand, subsequent study revealed that PHF2 plays an important role in adipogenesis. Phf2 knockout mice produced lipoatrophy in which adipocytes were decreased in size and number. It seemed to be limited to a young age; for example, in Klf5 knockout mice, atrophic changes in adipose tissue were abolished until 4 weeks of age (20). Although the reduced WAT phenotype in Phf2 knockout mice disappeared after the mice reached 8 weeks of age and Phf2 was systemically knocked out in these mice, this phenotype should be the result of a cell-autonomous mechanism because conditional knockout of Phf2 in primary SVCs obtained from Phf2fl/fl;Cre-ERT mice resulted in impaired adipogenesis. Moreover, PHF2 interacts with CEBPA, one of the master adipogenic regulators, and is recruited to CEBPRE. In addition, the lipoatrophic changes in Phf2 knockout mice were stronger in epididymal and subcutaneous WAT than in mesenteric WAT, suggesting that PHF2 may be a possible candidate factor that determines regional variations in adipogenesis reported previously (24). Although adipose tissue is well known to be involved in glucose homeostasis, there was no significant change in glucose metabolism in Phf2 knockout mice (Supplementary Fig. 3). There are two possible reasons for this result. First, 5 weeks of age is too young to develop insulin resistance, and second, only slight lipoatrophic change was observed in mesenteric WAT, the mass of which is strongly correlated with insulin resistance. It is meaningful to evaluate glucose metabolism in Phf2 knockout adult mice under the treatment of a high-fat diet to clarify whether PHF2 plays a role in diabetes.

It is well known that factors that increase cAMP, such as isobutylmethylxanthine, strongly accelerate adipogenesis.
Elevation of cAMP is known to lead to suppression of Wnt10b (26) and Sp1 (27), induction of C/EBPβ (28), and production of PPARγ ligands (29). Moreover, cAMP signaling is mediated by two major pathways, PKA and Epac (exchange proteins directly activated by cAMP), that synergistically induce adipogenesis (30). However, it is not fully understood how PKA regulates adipogenesis. PHF2 seems to be a possible candidate because it is reportedly activated through phosphorylation by PKA (5), and the current study shows that PHF2 stimulates adipogenesis. It seems that PHF2 was not transcriptionally regulated during adipogenesis (Supplementary Fig. 4). However, PHF2 might be activated by PKA-mediated phosphorylation by a cAMP inducer in the adipogenic differentiation cocktail.

On the other hand, the weight reduction of BAT was not observed in Phf2 knockout mice, although PKA and CEBPA were also reported to be necessary for the development of BAT (31). Recently, it was reported that brown adipocytes were differentiated from Myf5-positive precursor cells, which are more closely related to myoblasts rather than to the white preadipocytes (32). Thus, PHF2 might play a role in white preadipocytes but not in brown preadipocytes or myoblasts because the histone demethylases seem to be involved in differentiation in specific cell types. PHF2 reportedly interacts with ARID5B, which is necessary for the coactivator function of PHF2 (5). Because phenotypes of systemic Arid5b knockout animals have already been reported (33–35), it is worth comparing the phenotypes between Arid5b knockout and Phf2 knockout mice. Of note, most Arid5b knockout mice die within 24 h of birth, but some pups survive (33). The surviving Arid5b knockout animals show dramatically reduced body weight in neonates and adults. The WAT of Arid5b knockout mice weighed less than controls because of a reduction in the amount of lipid per cell. Because the phenotype of Arid5b knockout mice is close to that of Phf2 knockout mice, it is likely that PHF2 and ARID5B work together in multiple organs, including adipose tissue.

Transcriptional control of the adipocyte lineage has been studied extensively (21,36). However, little is known about the role of histone demethylases during adipogenesis, even though several studies have clarified the roles of histone deacetylase HDAC1 (37,38) and HDAC3 (39) and histone demethylase LSD1 (40) in adipogenesis. In the current study, we found that histone demethylase PHF2 plays an important role in adipogenesis in vivo. Notably, several studies reported the role of histone demethylation in cell differentiation; for example, LSD1 controls pituitary terminal cell-type differentiation (6), JMJD3, an H3K27me3 demethylase, potentiates epidermal differentiation (41); and JMJD1A, an H3K9 demethylase, potentiates smooth muscle cell differentiation (42). In general, histone demethylases may play roles in cell differentiation in specific cell types. We suggest that PHF2 may be a significant histone demethylase in adipogenesis.

Adipose tissue plays an essential role in energy homeostasis. In mammals, WAT stores excess energy as triglycerides from fatty acids imported from circulating lipoproteins. Moreover, recent studies established adipose tissue as an active endocrine organ that secretes various humoral factors called adipokines that work in various physiological pathways, such as feeding, insulin resistance, inflammation, and atherogenesis (43). Thus, clarifications of precise molecular mechanisms that control adipose tissue development should improve our understanding of the pathogenesis and pathophysiology of the metabolic syndrome, diabetes, and other metabolism-related diseases. In this study, PHF2 appears to be a novel molecule that controls adipogenesis in vivo. Modulation of enzymatic activities has been a good target of small molecules. In fact, some drugs that can modulate the activities of histone-modifying enzymes have been developed for clinical use (44–46). Thus, pharmacological modulation of the histone demethylase activity of PHF2 may be a new target in the treatment of human lipodystrophies or adipocyte hyperplasia in diet-induced obesity.

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Y.O. wrote the manuscript and analyzed data. F.O., T.M., I.T., S.K., and Y.I. designed the experiments. F.O. and Y.I. reviewed and edited the manuscript. K.I. and J.K. performed the microarray analyses. Y.I. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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