Loss of TDAG51 Results in Mature-Onset Obesity, Hepatic Steatosis, and Insulin Resistance by Regulating Lipogenesis

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Regulation of energy metabolism is critical for the prevention of obesity, diabetes, and hepatic steatosis. Here, we report an important role for the pleckstrin homology–related domain family member, T-cell death–associated gene 51 (TDAG51), in the regulation of energy metabolism. TDAG51 expression was examined during adipocyte differentiation. Adipogenic potential of preadipocytes with knockdown or absence of TDAG51 was assessed. Weight gain, insulin sensitivity, metabolic rate, and liver lipid content were also compared between TDAG51-deficient (TDAG51−/−) and wild-type mice. In addition to its relatively high expression in liver, TDAG51 was also present in white adipose tissue (WAT). TDAG51 was downregulated during adipogenesis, and TDAG51−/− preadipocytes exhibited greater lipogenic potential. TDAG51+/− mice fed a chow diet exhibited greater body and WAT mass, had reduced energy expenditure, displayed mature-onset insulin resistance (IR), and were predisposed to hepatic steatosis. TDAG51−/− mice had increased hepatic triglycerides and SREBP-1 target gene expression. Furthermore, TDAG51 expression was inversely correlated with fatty liver in multiple mouse models of hepatic steatosis. Taken together, our findings suggest that TDAG51 is involved in energy homeostasis at least in part by regulating lipogenesis in liver and WAT, and hence, may suggest that TDAG51 is involved in energy homeostasis at least in part by regulating lipogenesis in liver and WAT, and hence, may constitute a novel therapeutic target for the treatment of obesity and IR. Diabetes 62:158–169, 2013

With a dramatic rise in the prevalence of obesity and type 2 diabetes worldwide, there is an urgent need to better understand the genes involved in the metabolic processes underlying these conditions. Obesity results from an increase in white adipose tissue (WAT) mass, which occurs as a result of two processes, adipocyte hypertrophy and hyperplasia. The former process increases the size of adipocytes, and the latter is the result of an increase in mature adipocyte number through preadipocyte recruitment and adipocyte differentiation (1,2). The transcription factors peroxisome proliferator–activated receptor γ (PPARγ) (3,4) and CCAAT/enhancer–binding protein-α (C/EBPα) (5–7) play a crucial role during adipogenesis. In the later stages of terminal differentiation, proteins such as adipocyte–specific fatty acid–binding protein (aP2), adiponectin, and leptin are expressed (8). A fine balance between adipocyte hypertrophy and adipogenesis exists to prevent the formation of dysfunctional adipose tissue, since large cells are more likely to be insulin resistant (9,10) and therefore can influence adipose tissue metabolism. Despite significant progress over the last few years, additional genes and factors that influence adipogenesis, adipose tissue metabolism, and ultimately energy homeostasis still remain to be uncovered.

A recent microarray analysis of differentiating 3T3-L1 preadipocytes found T-cell death associated gene 51 (TDAG51), among other genes, to be induced within the first 8 h of adipogenesis (11) and was categorized in the same gene cluster as C/EBPβ and C/EBPδ, early adipogenic genes crucial for initiating the adipogenic gene expression cascade (12). Given the importance of the first 24 h in determining preadipocyte cell fate (13,14), this finding suggests that TDAG51 may be a novel candidate gene involved in adipogenesis. TDAG51 is a member of the pleckstrin homology–related domain family (human homolog PHLD1) (15) and was first demonstrated to be induced upon T-cell activation–mediated apoptosis in culture (16). However, in contrast to these in vitro findings, TDAG51-deficient (TDAG51−/−) mice displayed no apparent defects in T-cell apoptosis, number, or function (17). In addition to its PH-like domain, TDAG51 possesses distinct COOH-terminal proline-glutamine and proline-histidine repeats (18), which may be involved in its proapoptotic function in some cell types (19). TDAG51 has been suggested to play a role in tumorigenesis (20,21), and we have previously shown its expression in atherosclerotic lesions from hyperhomocysteinemic mice (22), although the precise physiological role of TDAG51 is not well understood. To date, the role of TDAG51 in adipogenesis and energy metabolism has not been examined.

We evaluated the protein expression pattern of TDAG51 in several tissues of wild-type (WT) C57BL/6 mice, which indicated that in addition to liver, which expresses the highest levels of TDAG51, WAT also expresses TDAG51. Here we report that TDAG51 expression was modulated during adipogenesis and that TDAG51−/− preadipocytes exhibited greater lipid accumulation under adipogenic conditions as compared with WT cells. TDAG51−/− mice displayed an age-related increase in whole-body adiposity and an overall metabolic profile resembling the metabolic syndrome. Furthermore, TDAG51 expression was inversely
correlated with fatty liver in several mouse models of hepatic steatosis. These findings suggest a previously unknown function for TDAG51 in promoting negative energy balance by regulating adipocyte and hepatic lipogenesis, thereby favoring energy expenditure.

**RESEARCH DESIGN AND METHODS**

**Western blotting and antibodies.** Cells and tissues were homogenized in 4× SDS lysis buffer, and protein lysates were subjected to immunoblotting as previously described (22). Antibodies were used to detect the following proteins: TDAG51, SREBP-1 (H06 for detection of precursor p125 and K10 for detection of mature p68), C/EBPα, PPARγ (Santa Cruz Biotechnology), Akt, pS473-Akt, S6K-1, fatty acid synthase (FAS), phosphoserine insulin receptor substrate 1 (phosphoserine IRS1), p2p, PPARγ, perilipin, glyceroldehyde-3-phosphate dehydrogenase (GAPDH) (Cell Signaling), IRS1, phosphotyrosine IRS1 (Invitrogen), and β-actin (Sigma).

**Isolation and culturing of primary cells.** Primary preadipocytes were cultured by isolating the subcutaneous fraction from mouse WAT. In brief, WAT was minced, collagenase (Sigma) digested, and centrifuged to separate the stromal-vascular fraction from the mature adipocyte fraction. The pellets were then plated and induced to differentiate in Dulbecco’s modified Eagle’s medium/F12 containing 2% FBS, 1 μg/ml insulin, 250 μmol/L dexamethasone, 0.5 μmol/L isobutylmethylxanthine, 60 μmol/L indomethacin, and 2.5 μmol/L rosiglitazone. Cells were extracted by the method of Li and Dryer (29). Neutral lipids were quantified as previously described (30). Lipid bands were visualized and scraped into counting vials for measuring radioactivity. Palmitate oxidation was determined as described previously (31). In brief, sodium palmitate (0.5 mmol/L) and [1-14C]-palmitate (0.5 μCi/ml; Perkin Elmer) were allowed to conjugate to 2% BSA for 2-3 h and then incubated with primary hepatocytes for 4 h, in the presence or absence of 10 nmol/L insulin. Radiolabeled CO2 was liberated by adding an equal volume of 1 mol/L acetic acid and trapped by the addition of benzethonium hydroxide (1 mol/L) over 90 min. The tube containing trapped 14CO2 was placed in a scintillation vial and counted.

**RESULTS**

**TDAG51 is expressed in preadipocytes and is down-regulated during adipogenesis.** TDAG51 protein expression was examined in various tissues from adult C57BL/6 mice. In addition to the liver and lung, which have abundant TDAG51 levels at the mRNA and protein level (15,17), WAT also expressed TDAG51 protein (Fig. 1A).

Previous microarray analysis during adipogenesis indicated that TDAG51 mRNA expression is modulated in early adipogenesis (11). Therefore, we carried out qRT-PCR analysis, which confirmed that TDAG51 mRNA was significantly induced in the first 2 h of adipogenesis followed by a sharp decline back to baseline expression (Fig. 1B). We next sought to evaluate the expression profile of TDAG51 at the protein level during adipocyte differentiation. TDAG51 protein expression was induced in the first 4 days post-stimulation, followed by a gradual decline after day 4, and diminished expression by day 14 of differentiation, when the vast majority of cells had accumulated large lipid droplets, as shown by Oil Red O staining (Fig. 1C, top). In addition, TDAG51 expression showed an inverse correlation with PPARγ expression during adipogenesis (Fig. 1C).

In support of the findings in 3T3-L1 cells, an inverse correlation between TDAG51 and PPARγ expression and/or adipogenesis was also observed in mouse embryonic fibroblasts induced to differentiate into adipocytes (Fig. ID). We next examined protein extracts from pooled human preadipocytes and their corresponding differentiated mature adipocytes, which also showed disappearance of TDAG51 in mature adipocytes (Fig. 1E). These findings indicate that TDAG51 is downregulated in differentiated lipid-laden adipocytes, suggesting that TDAG51 may play an important role in adipogenesis.
To reconcile the temporal difference in the expression of TDAG51 mRNA and protein during adipogenesis, 3T3-L1 cells were treated with cycloheximide to block protein translation. As shown in Fig. 1F, relative to other proteins that are crucial for adipogenesis, such as PPARγ and C/EBPβ, TDAG51 protein is considerably more stable. Absence of TDAG51 enhances lipid accumulation by altering early adipogenic gene expression during adipogenesis. In order to examine the role of TDAG51 in adipogenesis, primary stromal-vascular cells (SVCs) were isolated from WT and TDAG51−/− WAT and induced to differentiate. TDAG51−/− SVCs exhibited earlier and/or greater expression of adipogenic markers such as PPARγ2, aP2, FAS, perilipin, and C/EBPα on day 2 (Fig. 2A). By day 5, most of these adipogenic markers reached similar levels in WT and TDAG51−/− SVCs. However, despite the plateau in expression of these adipogenic markers, TDAG51−/− SVCs exhibited increased lipid accumulation, as determined by Oil Red O staining (Fig. 2B), and enhanced adiponectin secretion, a marker of mature adipocytes (Fig. 2C).

To complement these findings, both silencing/short interfering (si)RNA and short hairpin (sh)RNA targeting mouse TDAG51 were used to knockdown TDAG51.
expression in 3T3-L1 cells. Similar to TDAG51−/− SVCs, siTDAG51-transfected cells had significantly greater lipid accumulation compared with siControl-transfected cells (Supplementary Fig. 1A and B). An shTDAG51 construct was used, leading to a stable 30% knockdown in TDAG51 mRNA expression (Supplementary Fig. 1D) and resulted in significantly greater adiponectin secretion from shTDAG51 cells on day 3 (Supplementary Fig. 1D). Even this modest decrease in mRNA expression led to a striking decrease in TDAG51 protein (Supplementary Fig. 1D) and resulted in significantly greater adiponectin secretion from shTDAG51 cells on day 3 (Supplementary Fig. 1D). Expression of CEBPβ and C/EBPβ mRNA was higher within the first 8 h of differentiation but dropped off more quickly, and similarly mRNA expression of preadipocyte factor-1 (Pref-1) declined more quickly in shTDAG51 cells on day 1 as compared with shControl cells (Supplementary Fig. 1F). Analysis of late markers of adipogenesis, such as expression of PPARγ, aP2, and C/EBPα mRNA, indicated that they were expressed earlier (day 2) and to a greater extent in shTDAG51 cells (Supplementary Fig. 1G).

**TDAG51−/− mice develop increased adiposity due to reduced basal energy expenditure.** To study the effect of loss of TDAG51 on weight regulation in vivo, 8-week-old chow diet–fed male TDAG51−/− mice were monitored over 20 weeks and compared with age- and sex-matched heterozygous TDAG51+/− and WT mice. Body weight was not significantly different between genotypes at 8 weeks of age (Fig. 3A and Supplementary Fig. 2). However, over time, in comparison with WT mice, TDAG51−/− mice exhibited a significant increase in body weight (Fig. 3A), which was not observed for TDAG51+/− mice (Supplementary Fig. 2). There was no significant difference in tissue weights (Supplementary Fig. 3A and B) except for epididymal WAT (eWAT), where a significant increase was observed in TDAG51−/− mice (Fig. 3B). CT scans in 28-week-old mice showed that TDAG51−/− mice have a fivefold increase in total body WAT (Fig. 3C), with fat deposition in both the visceral and subcutaneous depots (Fig. 3C, top). A significant increase in adiposity was also observed in the bone marrow of TDAG51−/− mice (Supplementary Fig. 4A and B). Histological examination of the eWAT revealed that TDAG51−/− adipocytes were significantly enlarged compared with WT controls (Fig. 3D). In support of enhanced adipogenic and lipogenic potential observed in cultured preadipocytes (Fig. 2A–C), mRNA expression of several adipogenic genes (aP2, PPARγ, and C/EBPβ) was significantly increased in WAT from TDAG51−/− mice (Fig. 3E). These findings demonstrate that absence of TDAG51 enhances adipocyte lipid accumulation, leading to WAT expansion and increased body mass in TDAG51−/− mice.

Consistent with increased adiposity, TDAG51−/− mice had increased levels of systemic oxidative stress (TBARS) (Table 1), reaching levels similar to age-matched diet-induced obese mice (Supplementary Table 2), as well as increased plasma leptin and reduced adiponectin concentrations, compared with WT mice (Table 1). Plasma inflammatory markers (tumor necrosis factor-α [TNF-α], monocyte chemoattractant protein-1 [MCP-1], and IL12p70) tended to be higher in TDAG51−/− mice, but did not reach statistical significance (Supplementary Fig. 5A). Furthermore, staining of eWAT sections to detect macrophages revealed an increase in the number of crown-like structures in comparison with WT mice (Supplementary Fig. 5B). These findings are consistent with the notion that increased adiposity leads to adipose macrophage infiltration.
We next examined metabolic parameters using indirect calorimetry. Food consumption in WT and TDAG51<sup>2</sup>/<sup>2</sup> mice was not significantly different (Fig. 4A), and although there was a trend toward lower locomotor activity in TDAG51<sup>2</sup>/<sup>2</sup> mice, the difference did not reach statistical significance (Fig. 4B). However, TDAG51<sup>2</sup>/<sup>2</sup> mice exhibited a decrease in metabolic rate as indicated by a significant reduction in oxygen consumption (V<sub>O2</sub>) during both day and night hours (Fig. 4C). Core body temperature was also lower in TDAG51<sup>2</sup>/<sup>2</sup> mice during the night, which corresponded with their lower V<sub>O2</sub> (Fig. 4D).

We explored whether changes in BAT, an important regulator of thermogenesis in mice, could explain the difference in energy expenditure. Interscapular BAT from WT mice develops increased adiposity with enlarged adipocytes. A: Body weights at 8 and 28 weeks of age in male WT or TDAG51<sup>2</sup>/<sup>2</sup> mice (n = 7–8). B: eWAT weight at 28 weeks of age (n = 7). C: CT scans in 28-week-old WT and TDAG51<sup>2</sup>/<sup>2</sup> mice. Representative images are shown, with pink areas depicting the quantified WAT areas (n = 5, **P < 0.001). D: Perilipin stained, paraffin-embedded eWAT is shown in the top panel (bar, 250 μm). Adipocyte size is in μm<sup>2</sup> (n = 7). E: Adipogenic gene expression in WAT from 8-week-old mice, normalized to cyclophilin (n = 6). Data are expressed as the mean ± SEM. *P < 0.05 vs. WT. (A high-quality color representation of this figure is available in the online issue.)

![Graphs and images related to Figure 3 showing metabolic parameters and adipocyte size.]

**TABLE 1**

Plasma metabolic parameters in fasted 28-week-old WT and TDAG51<sup>2</sup>/<sup>2</sup> mice on chow diet

| Plasma Parameter  | WT       | TDAG51<sup>2</sup>/<sup>2</sup> |
|-------------------|----------|-------------------------------|
| ALT (units/L)     | 33.3 ± 3.5 | 32.3 ± 3.8                   |
| TBARS (μmol/L)    | 0.15 ± 0.1 | 3.75 ± 1.3*                  |
| Chol-E (mg/dL)    | 92.3 ± 5.7 | 94.8 ± 2.6                   |
| FFA (mmol/L)      | 0.65 ± 0.03 | 0.81 ± 0.06*                |
| TG (mg/dL)        | 48.5 ± 5.4 | 49.0 ± 2.2                   |
| Adiponectin (μg/mL) | 11.9 ± 0.8 | 6.3 ± 0.6**                |
| Leptin (ng/mL)    | 1.9 ± 0.5  | 14.7 ± 2.6**                 |

Data shown in the table represent means ± SEM. ALT (n = 6–7); TBARS (n = 6–7); cholesterol-E (Chol-E) (n = 7–8); FFA (n = 7–8); TGs (n = 7–8); adiponectin (n = 7–8); leptin (n = 7–8). *P < 0.05, between WT and TDAG51<sup>2</sup>/<sup>2</sup>. **P < 0.01, between WT and TDAG51<sup>2</sup>/<sup>2</sup>. 162 DIABETES, VOL. 62, JANUARY 2013 diabetes.diabetesjournals.org
and TDAG51−/− mice was examined to assess cell morphology, and we found an overall increase in lipid droplet size in BAT from TDAG51−/− mice (Fig. 4E). We next examined expression of thermogenic and mitochondrial genes in BAT from WT and TDAG51−/− mice. No significant differences were found in the expression of UCP1, CIDEA, PGC1α, CPT1b, or PPARγ, and we did not detect any differences in the expression of components of the electron transport chain (Supplementary Fig. 6A). Furthermore, no overall difference in UCP-1 immunostaining of BAT paraffin sections was observed between genotypes (Supplementary Fig. 6B). To assess mitochondrial morphology and

FIG. 4. Energy expenditure is reduced in TDAG51−/− mice. A: Food intake at 20 weeks of age (n = 7). B: Total locomotor activity in 20-week-old mice during day and night hours (n = 7). NS, not significant. VO2 (oxygen uptake) (C) and core body temperature (D) in 20-week-old mice (n = 7). E: Hematoxylin-eosin–stained BAT from 28-week-old mice (Bar, 50 μm). F: Gene expression analysis of thermogenic and mitochondrial genes in BAT (n = 6–7). Data are expressed as the mean ± SEM. *P < 0.05 and **P < 0.01 vs. WT. (A high-quality color representation of this figure is available in the online issue.)
size, BAT was further examined using transmission electron microscopy; however, no striking differences in mitochondria shape, size, or cristae were observed between genotypes (Supplementary Fig. 6C). Given that TDAG51 expression is ~90% lower in BAT compared with WAT of WT mice (Supplementary Fig. 6D), it is possible that BAT function in TDAG51−/− mice would not be directly affected by the absence of TDAG51. These findings suggest an increase in ectopic lipid deposition in BAT; however, BAT function does not appear to be significantly affected.

**TDAG51−/− mice develop age-associated insulin resistance.** Fasting plasma glucose and insulin levels were significantly elevated in TDAG51−/− mice at 28 weeks of age (Fig. 5A and B). In young 8-week-old WT and TDAG51−/− mice, an age where whole-body, liver, and WAT weights were indistinguishable (Fig. 3A and Supplementary Fig. 7A), GTTs and ITTs revealed no differences between genotypes (Supplementary Fig. 7B and C). However, at 28 weeks of age, when TDAG51−/− mice exhibited a significant increase in WAT and body weight, TDAG51−/− mice were also significantly more glucose intolerant (Fig. 5C) and insulin resistant (Fig. 5D) compared with WT mice. The efficiency of glucose uptake in various tissues was examined using PET scanning, which revealed a significant decrease in glucose uptake in skeletal muscles and WAT of TDAG51−/− mice (Fig. 5E). WAT from TDAG51−/− mice also showed decreased insulin receptor signaling, as indicated by reduced tyrosine (Y608) phosphorylation of IRS-1 and increased phosphorylation on serine (S612), which resulted in hepatic TG accumulation due to impaired insulin receptor signaling (Fig. 5F). To determine whether these results were secondary to the insulin-resistant phenotype or intrinsic to the loss of TDAG51, primary hepatocytes from 6-week-old WT and TDAG51−/− mice, which displayed no differences in body weight or metabolic phenotype, were examined. TDAG51−/− hepatocytes responded normally to insulin (Supplementary Fig. 8B) but had enhanced basal and insulin-stimulated lipogenesis (rate of newly synthesized fatty acid incorporation into TG) (Fig. 7A). Palmitate oxidation, on the other hand, was not different between genotypes when examined basally or upon insulin stimulation in primary hepatocytes (Fig. 7B). Furthermore, levels of PPARα, a transcription factor involved in fatty acid catabolism, was not affected by the absence of TDAG51 in liver tissue (Supplementary Fig. 8A). Consistent with enhanced de novo lipogenesis, primary hepatocytes from TDAG51−/− mice expressed higher mRNA levels of the SREBP-1c target genes glucokinase (GK) and acetyl CoA carboxylase 1 (ACC1) (Fig. 7C). These results in cultured hepatocytes are in full agreement with our findings in livers from TDAG51−/− mice, suggesting that deletion of TDAG51 promotes lipogenesis at least in part through increased SREBP-1 activation and its downstream target gene expression.

**DISCUSSION**

Dysregulation of the pathways involved in adipogenesis, lipid metabolism, and energy homeostasis can lead to the development of obesity, hepatic steatosis, diabetes, and dyslipidemia. Here, we present novel evidence that TDAG51 plays a significant role in the regulation of energy and lipid metabolism. First, the findings of this study show for the first time that TDAG51 is expressed in WAT and that its protein expression is modulated during adipogenesis. Given that TDAG51 protein expression is minimal or absent in mature mouse and human adipocytes, TDAG51 may be a usefulpreadipocyte marker. Second, deletion of TDAG51 enhances lipid accumulation during adipogenesis and accelerates fatty acid/TG synthesis in...
FIG. 5. *TDAG51*+/− mice develop age-associated IR. Fasting glucose (A) and fasting insulin (B) concentrations in 28-week-old male mice (n = 7–8). C: GTT after an overnight fast and the area under the curve (AUC) in the right panel (n = 4). D: ITT after a 6-h fast and the AUC (right) (n = 6–7). E: Intravenous injection of 2-FDG followed by a PET scan. Quantification of 2-FDG in various tissues (n = 5). PET scan images from representative mice are shown in the right panel. F: Mice were injected with insulin after a 6-h fast, and eWAT was collected 15 min later for Western blotting examining insulin receptor signaling (n = 5 mice). G: Pancreatic islets taken at the same magnification, where red staining represents insulin and green depicts glucagon. Data are expressed as the mean ± SEM. *P < 0.05 and **P < 0.01 vs. WT.
primary hepatocytes. Third, the absence of TDAG51 leads to an age-associated increase in whole-body adiposity and hepatic TG accumulation, and decreases metabolic rate and insulin sensitivity in standard chow diet–fed WT mice. Notably, the phenotype observed in TDAG512/2 mice resembles several aspects of high-fat diet–fed WT mice. Finally, TDAG51 protein expression is inversely correlated with hepatic steatosis.

The age-dependent increase in adiposity, hepatic TG accumulation, and systemic IR in TDAG512/2 mice are
SREBP-1 target gene expression was observed in phenotype observed in older and oxidative stress (35,36), which is consistent with the diabetes. Consistent livers, the absence of TDAG51 likely leads to hepatic de novo lipogenesis (32) these mice. Given that SREBP-1 is a master regulator of mice may be contributing to the systemic IR phenotype in the expansion of WAT, ectopic lipid accumulation, and lipidogenesis. Excessive accumulation of TG can culminate tributable to a decrease in metabolic rate and increased suggestive of a chronic and cumulative effect, likely attributable to a decrease in metabolic rate and increased lipogenesis. Excessive accumulation of TG can culminate in the expansion of WAT, ectopic lipid accumulation, and eventually IR in combination with chronic inflammation and oxidative stress (35,36), which is consistent with the phenotype observed in older TDAG51/-/- mice. Moreover, when cells become insulin resistant, not all pathways are blunted like domain, which appears to be a likely candidate for functional significance in the context of cell signaling and metabolism. For instance, a recent study identified a novel PH domain–containing protein involved in the regulation of adipocyte insulin signaling (43). Indeed, TDAG51 was identified as a specific IGF-1 target gene in NIH-3T3 fibroblasts and was shown to mediate the effects of IGF-1 on cell survival (44). Furthermore, previous studies suggest that extracellular signal–related kinase (ERK) and p38 mitogen-activated protein kinase (p38 MAPK) signaling can modulate TDAG51 expression (44,45). However, the exact cellular mechanisms by which IGF-1, ERK, or p38 MAPK induces TDAG51 expression are unknown.

FIG. 7. TDAG51/-/- primary hepatocytes exhibit greater lipogenic potential. A: Basal and insulin-stimulated (100 nmol/L) 3H-acetate incorporation into triglycerides (TG) in primary hepatocytes from 6-week-old mice treated with vehicle or insulin. B: 14C-Palmitate oxidation in primary hepatocytes from 6-week-old mice treated with vehicle or insulin. C: qRT-PCR analysis examining mRNA expression of GK and ACC1 in cultured primary hepatocytes. Data are expressed as the mean ± SEM, n = 3. *P < 0.05 and **P < 0.01 vs. WT.

TDAG51 has several important domains, including a PH-like domain, which appears to be a likely candidate for functional significance in the context of cell signaling and metabolism. For instance, a recent study identified a novel PH domain–containing protein involved in the regulation of adipocyte insulin signaling (43). Indeed, TDAG51 was identified as a specific IGF-1 target gene in NIH-3T3 fibroblasts and was shown to mediate the effects of IGF-1 on cell survival (44). Furthermore, previous studies suggest that extracellular signal–related kinase (ERK) and p38 mitogen-activated protein kinase (p38 MAPK) signaling can modulate TDAG51 expression (44,45). However, the exact cellular mechanisms by which IGF-1, ERK, or p38 MAPK induces TDAG51 expression are unknown.

PH domains play an important role in recruiting target proteins to cell membranes through binding interactions with phospholipids such as phosphatidylinositol (3-5)-triphosphate (PIP3) and phosphatidylinositol (4,5)-bisphosphate (PIP2) (46,47). Interestingly, TDAG51 has a high stringency PIP3-binding site in its PH-like domain, as predicted by ScanSite (48). Furthermore, the PH-like domain of TDAG51 exhibits a high degree of homology to that of phosphatidylinositol 3-kinase (PI3K) enhancer (PIKE), as predicted by the Protein Homology/Analog Y Recognition Engine (Phyre2) (49). Interestingly, PIKE has recently been implicated in playing a role in adipogenesis, WAT regulation, and insulin sensitivity, and in contrast to TDAG51/-/- mice, PIKE/-/- mice are protected from diet-induced weight gain and IR (50). Thus, we are currently exploring whether the PH-like domain of TDAG51 is indeed mediating its effects on the regulation of lipogenesis and whole-body energy metabolism and whether the PI3K/Akt pathway may be involved.

Collectively, the results of our study illustrate that TDAG51 plays an important role as a regulator of carbohydrate and lipid metabolism, at least in part through regulating SREBP activity, which may contribute to the phenotypes observed with the metabolic syndrome. Understanding this newly identified cellular factor in lipid metabolism and adipogenesis as well as its precise regulation under normal and pathophysiologic conditions will be crucial for the development of effective treatments against diabetes and obesity.
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No potential conflicts of interest relevant to this article were reported.

S.B. performed experiments, collected and interpreted data, and wrote the manuscript. S.L., M.D.F., R.P., and E.G.L. performed experiments, contributed to data interpretation, and edited the manuscript. H.J. and R.J.F. assisted with experiments. K.N.M., G.R.S., and R.C.A. contributed to data interpretation and discussion and reviewed the manuscript. R.C.A. 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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