A mouse model of conditional lipodystrophy

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Lipodystrophies are syndromes of adipose tissue degeneration associated with severe defects in lipid and glucose homeostasis. We report here the generation and analysis of *Pparg^{ldi}*, a targeted allele that confers conditional dominant lipodystrophy in mice. The *Pparg^{ldi}* allele was generated by insertion of the Tet activator (tTA) and a tTA-regulated *Flag-Pparg1* transgene into the *Pparg* gene. Unexpectedly, tTA elicits mild lipodystrophy, insulin resistance, and dyslipidemia, and the *Flag-PPAR γ 1* transgene surprisingly exacerbates these traits. Doxycycline can both completely prevent and reverse these phenotypes, providing a mouse model of inducible lipodystrophy. Embryonic fibroblasts from either *Pparg^{ldi/+}* or the phenotypically similar *aP2-nSrebp1c* (*Sr*) transgenic mice undergo robust adipogenesis, suggesting that neither strain develops lipodystrophy because of defective adipocyte differentiation. In addition, *Pparg^{ldi/+}* adipose tissue shares extensive gene expression aberrations with that of *Sr* mice, authenticating the phenotype at the molecular level and revealing a common expression signature of lipodystrophic fat. Thus, the *Pparg^{ldi/+}* mouse provides a conditional animal model for studying lipodystrophy and its associated physiology and gene expression.

doxycycline | adipocyte | insulin resistance

Generalized and partial lipodystrophies are rare syndromes of heritable or acquired adipose tissue degeneration and misdistribution, often accompanied by severe defects in lipid and glucose homeostasis (reviewed in refs. 1 and 2). Genetic causes have been identified for several familial forms of the disease in humans and include mutations in the *AGPAT2*, *BSCL2* (Seipin), *LMNA* (Lamin A), *ZMPSTE24* (a lamin-processing protease), *PPARG*, or *AKT2* genes. Acquired lipodystrophies are either idiopathic or arise because of immunological disorders, such as autoimmunity or complement activation. An increasingly prevalent type of acquired lipodystrophy occurs in HIV-infected patients subject to prolonged protease inhibitor therapy (3). This lipodystrophic syndrome, whose molecular etiology is unclear, is typified by loss of s.c., peripheral, facial, and gluteal fat and accumulation of excess fat around the neck and trunk, along with insulin resistance, type II diabetes, and dyslipidemia.

Several mouse models have been developed and characterized to date for the study of lipodystrophy and its consequences. Mice with deliberate subversion of adipocyte differentiation or viability include: *aP2-DTA* mice, in which lipoatrophy was engineered by adipocyte-specific expression of diphtheria toxin (4); *A-ZIP/F* mice, which were rendered lipoatrophic by artificial interference with the adipogenic C/EBP transcription factors (5); and *FAT-ATTAC* mice, which undergo transient lipoatrophy after temporal induction of adipocyte apoptosis (6). These models are useful for exploring the outcomes of lipodystrophy and general treatment modalities. Models in which lipodystrophy arose expectedly or serendipitously because of mutation or misexpression of endogenous genes include the following: *aP2-nSrebp1c* mice (*Sr*), which express a truncated, constitutively active *Srebp-1c* transgene in adipocytes, and whose lipodystrophic phenotype is neither expected nor mechanistically understood (7); *fld* ("fatty liver dystrophy") mice, which suffer from severe lipoatrophy because of spontaneous mutations in the

Lpin1 gene (8); and several knockout mouse strains with varying degrees of lipodystrophy, including mice deficient for *Lmna* (9), *Zmpste24* (10), ribosomal S6 kinase (*Rsk2*) (11), *Irs1/Irs3* double-knockout mice (12), and adipocyte-specific *Pparg*-null mice (13–15). These mouse models are useful for studying both the etiology and consequences of lipodystrophy.

Here, we report a mouse model of lipodystrophy, which is the serendipitous outcome of a genetically engineered allele of peroxisome proliferator-activated receptor γ (PPAR γ), termed *Pparg^{ldi}*. The *Pparg^{ldi}* allele confers dominantly heritable lipodystrophy, which shares extensive anatomic, metabolic, and gene expression properties with *Sr* transgenic mice. The pathology is caused by unexpected combined effects of a knocked-in Tet-activator protein, tTA (16), and a Tet-regulated *Flag-Pparg1* transgene and is completely inhibited by doxycycline, making the *Pparg^{ldi/+}* mouse a unique model of conditional lipodystrophy. We further show that neither *Pparg^{ldi/+}* nor *Sr* fibroblasts exhibit major adipogenic defects, suggesting that both arise subsequent to normal adipocyte differentiation. The *Pparg^{ldi/+}* mouse offers an ideal inducible platform for studying both systemic and adipose tissue-specific attributes of lipodystrophy.

Results

The *Pparg^{ldi}* Allele. The *Pparg^{ldi}* allele was originally designed for substituting the endogenous *Pparg* gene with recombinant, inducible PPAR γ . It was generated by homologous recombination of the second coding exon of *Pparg* with a cassette encoding the tetracycline-regulated transactivator (*tTA*; ref. 16), *Pgk-Neo*, and *Tet* operator-controlled *Flag-Pparg1* cDNA (Fig. 1*A* and *B*). The predicted fusion transcript between the 5' portion of the endogenous *Pparg* gene and the knocked-in *tTA* (*AF1-IRES-tTA*) was abundantly expressed in adipose tissue of *Pparg^{ldi/+}* mice, as was the tTA protein (Fig. 1*C–E*). An ≈ 1.5 -kb transcript, corresponding to *Tet operator-driven Flag-Pparg1* cDNA, was modestly expressed in *Pparg^{ldi/+}* adipose tissue (Fig. 1*C*). However, expression of the Flag-PPAR γ 1 protein in adipose tissue was below the detection threshold (Fig. 1*F*). Both *tTA* and *Flag-Pparg1* were poorly expressed in other *Pparg*-expressing tissues of *Pparg^{ldi/+}* mice, such as placenta and liver (data not shown). Interestingly, endogenous PPAR γ 2, but not the PPAR γ 1 isoform, was drastically reduced in the heterozygous fat pads, despite integrity of the companion WT *Pparg* allele (Fig. 1*F*).

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Abbreviations: BAT, brown adipose tissue; dox, doxycycline; EFP, epididymal fat pad; PPAR γ , peroxisome proliferator-activated receptor γ ; *Sr*, *aP2-nSrebp1c*; tTA, tetracycline-regulated transactivator; WAT, white adipose tissue.

Data deposition: Microarray data have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE9132).

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Fig. 1. The *Pparg^{ldi}* allele. (A) The *Pparg^{ldi}* targeting cassette contains, from 5' to 3', the following: an internal ribosome entry site (IRES), the Tet activator gene, *tTA* (15), *Pgk-neo*, a synthetic polyA module, and a Tet operator (*TetO*)-driven *Flag-Pparg1* cDNA. The cassette was targeted into the second coding exon of the mouse *Pparg* gene via homologous recombination, as shown. (B) Southern blots of BamHI-digested genomic DNA were hybridized to 5' and 3' probes external to the genomic arms of the targeting construct (see A). Correctly targeted *Pparg^{ldi}* ES cell clones (T) exhibit the predicted restriction fragment lengths. (C) A Northern blot of two WT (+/+) and two *Pparg^{ldi}* epididymal WAT pads was hybridized to the 5' portion of *Pparg* cDNA. *Pparg^{ldi}* WAT expresses both WT *Pparg* and an abundant fusion transcript of the 5' part of *Pparg*, IRES, and *tTA* (*AF1-IRES-tTA*), as well as modest levels of an ≈1.5-kb transcript, corresponding to the transgenic *Flag-Pparg1*; expression and identity of the latter transcript were confirmed by RNase protection analysis (data not shown). (D) A *tTA* probe corroborates the identity of the *AF1-IRES-tTA* transcript in *Pparg^{ldi}* WAT and BAT. (E) Anti-VP16 antibodies detect ample *tTA* in *Pparg^{ldi}* WAT and BAT protein extracts (*tTA* is a Tet repressor-VP16 fusion protein). BAT exhibits an additional, nonspecific reactive band (NS). (F) PPARγ isoforms in WT and *Pparg^{ldi}* WAT. A SDS/7.5% PAGE Western blot was reacted with a custom-made anti-PPARγ antibody. Identities of PPARγ1, Flag-PPARγ1, and PPARγ2 proteins were determined based on migration patterns of *in vitro*-translated counterparts (IVT) (PPARγ2 data not shown). Flag-PPARγ1 protein could not be detected in *Pparg^{ldi}* WAT extracts, whereas PPARγ2 was suppressed.

***Pparg^{ldi}* Mice Exhibit Severe, Doxycycline-Treatable Lipodystrophy.** Unexpectedly, heterozygous *Pparg^{ldi}* mice derived from three independent ES cell clones exhibited overt, dominantly inherited lipodystrophy. Anatomical and histological aspects of this phe-

Fig. 2. The histological *Pparg^{ldi}* phenotype. (A) A characteristic buffalo hump in a 5-day-old *Pparg^{ldi}* pup (arrow). (B and C) BAT of WT (+/+) and *Pparg^{ldi}* mice. *Pparg^{ldi}* BAT exhibits markedly enlarged lipid droplets with unilocular vs. multilocular deposition (C vs. B). (D and E) WT and *Pparg^{ldi}* skin. Subdermal fat is eliminated in *Pparg^{ldi}* mice. (F and G) Epididymal fat pads (EFP) of WT and *Pparg^{ldi}* mice. White adipose mass is substantially decimated in *Pparg^{ldi}* mice. (H–K) Histology of WT and *Pparg^{ldi}* WAT. (I) A domain of marked adipocyte hypertrophy in the *Pparg^{ldi}* tissue. (J) Another domain in the same EFP with minuscule cells, fragmented lipid droplets, and marked leukocyte infiltration. (K) A higher power micrograph of J Inset, with clear leukocytes and fragmented lipid droplets. (L–O) Complete phenotypic reversal in *Pparg^{ldi}* mice treated with dox from gestation onwards. Shown are BAT (L), skin (M), EFP (N), and WAT (O); compare with untreated mutants in C, E, G, I–J, respectively. All tissue specimens, except in A, are from 10-wk-old male cohorts. (Scale bars: B, C, H–J, L, and O, 50 μm; D, E, and M, 250 μm; K, 10 μm.)

notype included: (i) “buffalo humps” (Fig. 2A), comprising swollen interscapular fat pads due to hypertrophy and unilocular lipid deposition in mutant brown adipocytes (compare Fig. 2C with Fig. 2B); (ii) complete depletion of s.c. adipocytes (compare Fig. 2E with Fig. 2D); (iii) severely reduced size of gonadal white adipose tissue (WAT) (Fig. 2G vs. Fig. 2F); (iv) irregular shapes and sizes of residual adipocytes, ranging from overtly hypertrophic (compare Fig. 2I with Fig. 2H) to minuscule cells (Fig. 2J); and (v) fibrotic WAT stroma filled with fragmented lipid droplets and leukocytes (Fig. 2K). Importantly, all of these defects were completely prevented by continuous administration of doxycycline (dox) in the drinking water from midgestation onward (Fig. 2L–O). Likewise, a 6-wk dox treatment of pubertal *Pparg^{ldi}* mice fully reversed all of their defects, with the exception of WAT, whose histology was entirely corrected without regaining normal size (data not shown).

Pparg^{ldi} mice displayed typical metabolic sequelae of lipodystrophy. These included the following: (i) early onset increases in the levels of circulating triglycerides, cholesterol, and free fatty acids (Fig. 3A–C); (ii) hyperglycemia in prepubertal *Pparg^{ldi}* males ($P < 0.05$), which normalized later in life, with sporadic, transient hyperglycemia in individual mutant mice (Fig. 3D); (iii) hyperinsulinemia (Fig. 3E), pancreatic islet hyperplasia (compare Fig. 3G with Fig. 3F), and severe glucose intolerance (Fig.

Fig. 3. The metabolic *Pparg*^{ldi/+} phenotype. (A–E) Levels of plasma triglycerides (A), free fatty acids (B), cholesterol (C), glucose (D), and insulin (E) in matched, 6-h-fasted WT (white bars; *n* = 6–10) and *Pparg*^{ldi/+} cohorts (black bars; *n* = 6–10). Values are presented for 4-wk-old females and a longitudinal analysis of males aged 4–12 wk. Asterisks designate statistically significant differences (*P* < 0.05) between *Pparg*^{ldi/+} and WT mice. (F and G) Pancreatic islet hyperplasia in *Pparg*^{ldi/+} mice. (H) Severe glucose intolerance in *Pparg*^{ldi/+} (*n* = 5) as compared with WT mice (*n* = 5). (I) Hepatomegaly in *Pparg*^{ldi/+} mice. (J and K) Hepatic histology of WT and *Pparg*^{ldi/+} mice. *Pparg*^{ldi/+} hepatocytes are hypertrophic, and contain large unstained vacuoles, but do not display the prototypic lipid droplet accumulation of steatotic livers. (L and M) Dox treatment circumvents hypertriglyceridemia and hyperinsulinemia in *Pparg*^{ldi/+} mice. Values were measured in untreated 5.5-wk-old WT and *Pparg*^{ldi/+} cohorts (–) compared with matched cohorts exposed to dox from midgestation onwards (+). (N) Aldehyde Fuchsin-stained pancreas from a 16-month-old *Pparg*^{ldi/+} male demonstrates rich insulin content in β -cells of aged mutants. Tissue specimens were collected from 10-wk-old male cohorts, except in N. (Scale bars: F, G, and N, 100 μ m; I, 1 cm; J and K, 50 μ m.)

3H); and (iv) a 60–150% increase in liver size (Fig. 3I) accompanied by hepatocyte hypertrophy and vacuolization, but not the prototypic lipid droplet accumulation observed in classical steatosis (Fig. 3J and K). Like the histological defects, all metabolic anomalies of *Pparg*^{ldi/+} mice were completely reversed by dox treatment (Fig. 3L and M and data not shown). Thus, the Tet-regulated components of the *Pparg*^{ldi} allele, and not structural interference with endogenous *Pparg*, are responsible for the unexpected lipodystrophic phenotype in its entirety.

Despite dyslipidemia and severe insulin resistance throughout life, *Pparg*^{ldi/+} mice developed neither bona fide steatosis nor type II diabetes. Mice as old as 12–18 months exhibited hyperplastic yet intact and insulin-laden pancreatic islets (Fig. 3N),

Fig. 4. tTA causes mild lipodystrophy with modest metabolic anomalies. (A) The *Pparg*^{tTA} allele contains an exact replica of the IRES, tTA, *Pgk-neo*, and synthetic polyA modules of the *Pparg*^{ldi} allele, targeted to the same location within the second coding exon of the *Pparg* gene. (B) Southern blots of BamHI-digested genomic DNA hybridized to 5' and 3' external probes. Correctly targeted *Pparg*^{tTA/+} ES cell clones (T) exhibit the digestion patterns predicted in A. (C) A Northern blot of two WT (+/+) and two *Pparg*^{tTA/+} EFPs was hybridized to the 5' *Pparg* cDNA probe, as described in Fig. 1C. *Pparg*^{tTA/+} WAT expresses the same AF1-IRES-tTA fusion transcript seen in *Pparg*^{ldi/+} fat. (D–K) BAT (D and E), skin (F and G), EFPs (H and I), and WAT (J and K) from 10-wk-old WT (D, F, H, and J) and *Pparg*^{tTA/+} (E, G, I, and K) males. BAT exhibits approximately the same phenotype of *Pparg*^{ldi/+} mice (see Fig. 2C), whereas subdermal fat and WAT exhibit a markedly attenuated phenotype (compare with Fig. 2D, I, and J. Scale bars: D, E, J, and K, 50 μ m; F and G, 250 μ m.) (L and M) Fasting triglyceride (L) and insulin (M) levels in 5.5-wk-old WT, *Pparg*^{tTA/+}, and *Pparg*^{ldi/+} males (*n* = 5–6 mice). All cohorts were born within the same week and assayed simultaneously. Notice the intermediate hypertriglyceridemic and hyperinsulinemic phenotypes of *Pparg*^{tTA/+} compared with *Pparg*^{ldi/+} mice. * and **, levels of both analytes are significantly different in each strain compared with the other two (*P* < 0.05).

with hyperinsulinemia and normoglycemia (data not shown). These observations suggest that steatosis and diabetes are not automatic complications of chronic hyperlipidemic or hyperinsulinemic burdens, respectively, and the development of each requires additional physiological factors.

Pathogenic Determinants of the *Pparg*^{ldi} Allele. To dissect the causes of the *Pparg*^{ldi} phenotype, we generated and analyzed a targeted

Fig. 5. Unperturbed adipogenesis of *Pparg*^{*ldi*+/+} and *Sr* embryonic fibroblasts. Primary fibroblasts from embryonic day 13.5 WT, *Pparg*^{*ldi*+/+}, and *Sr* embryos were subject to adipogenic differentiation. (A) On the 12th day of differentiation, cultures were stained with Oil-red-O and were photographed at low (Upper) and high magnification (Lower), revealing comparable lipid accumulation between both *Pparg*^{*ldi*+/+} and *Sr* cells and the corresponding WT cultures. (B) RNA was extracted from cultures throughout differentiation and was analyzed by Northern blot hybridization to *Pparg*, *Fabp4* (aP2), and *Adrb3* (β3-adrenergic receptor). *Pparg* levels are more substantially decreased in *Pparg*^{*ldi*+/+} fibroblasts, reflecting gene dosage reduction to a single WT copy. Although nominal marker expression levels are slightly reduced in the mutant fibroblasts, induction kinetics is similar between WT and mutant cells, demonstrating normal progress of adipogenesis. Ribosomal RNA (rRNA) levels are shown for normalization.

allelic variant, *Pparg*^{*tTA*}, which expressed the exact *IRES-tTA* knockin configuration as in *Pparg*^{*ldi*} but lacked the *TetO*-driven *FlagPparg1* module (Fig. 4A–C). Surprisingly, mice derived from two independent *Pparg*^{*tTA*+/+} ES clones manifested histological and metabolic characteristics of lipodystrophy, albeit substantially more modest than *Pparg*^{*ldi*+/+} mice. Specifically, (i) brown adipocytes were pale, unilocular, and hypertrophic (Fig. 4D and E), yet not to the overt extent of buffalo humps; (ii) s.c. fat was reduced, but not eliminated (Fig. 4F and G); (iii) the gonadal fat pads were smaller than those of WT mice (Fig. 4H and I), although not as severely as in *Pparg*^{*ldi*+/+} mice, and exhibited neither adipocyte hypertrophy nor fibrosis and almost no leukocyte infiltration (Fig. 4J and K); (iv) triglyceride and insulin levels were elevated, albeit to a significantly lesser extent than in *Pparg*^{*ldi*+/+} mice (Fig. 4L and M); and (v) hepatomegaly was modest (data not shown). Dox treatment completely suppressed these pathologies (data not shown), suggesting that basal lipodystrophy results from promiscuous transcriptional activity of tTA, rather than generalized transcription cofactor titration by its VP16 moiety (17). Most critically, the markedly attenuated *Pparg*^{*tTA*+/+} phenotype indicated that the *Flag-Pparg1* transgene is responsible for the dramatically exacerbated pathology of *Pparg*^{*ldi*+/+} mice. Resequencing of the *Pparg*^{*ldi*} targeting construct confirmed that apart from the engineered Flag epitope in its N terminus, this cDNA was 100% identical to WT *Pparg1*, and, thus, its pathogenic activity is not due to accidental mutation.

***Pparg*^{*ldi*+/+} and *Sr* Fibroblasts Undergo Normal Adipogenesis.** Congenital lipodystrophy is commonly ascribed to defective adipocyte differentiation (1, 2, 5, 7, 8). Adipogenic disorders clearly underlie lipodystrophy in both A-ZIP/F-1 transgenic mice (5) and *Lpin1*-deficient *Fld* mouse (8). However, such an etiology is

Fig. 6. Overlapping gene expression aberrations in *Pparg*^{*ldi*+/+} and *Sr* fat. Shown is a Venn diagram depicting the numbers of overlapping and strain-specific genes that are differentially expressed by >2.5-fold in *Pparg*^{*ldi*+/+} (*Ldi*) and/or *Sr* mice. (Inset) The number of genes that change by at least 7-fold in each strain partitioned according to their overlap with the reciprocal strain (7-fold, 2.5- to 7-fold, <2.5-fold). See SI Table 1 for the full list of genes in the 7-fold inset.

harder to reconcile with the normal elaboration of *Pparg*^{*ldi*+/+} brown adipose tissue (BAT), as hypertrophic and dysfunctional as it eventually becomes, or with the presence of WAT in these mice, as residual as it may be. Similar considerations apply to the etiologically distinct yet phenotypically similar *Sr* mice (7). Indeed, *in vitro* adipogenesis of both *Pparg*^{*ldi*+/+} and *Sr* embryonic fibroblasts was consistently normal, as judged by both lipid accumulation and expression of adipogenic markers in at least five independent preparations of each (Fig. 5). These observations suggest that lipodystrophy in these two mouse models may not be the result of perturbed adipocyte differentiation *per se*.

Overlapping Gene Expression Aberrations in *Pparg*^{*ldi*+/+} and *Sr* Adipose Tissue. To better define the *Pparg*^{*ldi*+/+} mouse in relation to other models of lipodystrophy, we compared the gene expression profiles of *Sr* and *Pparg*^{*ldi*+/+} gonadal fat pads. These profiles combine genuine changes in gene regulation with changes that arise from the skewed cellular demographics of lipodystrophic fat pads, i.e., richer stromal cell populations and a decrease in the relative adipocyte fraction. By averaging the reduction in multiple adipocyte-specific genes and the increase in prototypic markers of endothelial cells and leukocytes we estimated that adipocyte abundance is reduced by ≈2.5-fold in the mutant tissues, whereas different stromal cell types are enriched 2.5- to 4-fold. Differentials that exceed these values are therefore more likely to represent regulatory changes than aberrant tissue composition. A staggering 1,006 and 1,111 transcripts changed by >2.5-fold in WAT of *Pparg*^{*ldi*+/+} and *Sr* mice, respectively, relative to their WT counterparts (Fig. 6). Of these, 322 differentials were shared by both mouse strains. Moreover, 74 and 115 genes changed by >7-fold in *Pparg*^{*ldi*+/+} and *Sr* fat, respectively, 24 of which were shared by both strains, and an additional 46 genes changed by >7-fold in one strain and between 2.5- and 7-fold in the other. This substantial overlap in robust gene expression aberrations points to widespread molecular similarities between the two strains and reveals a gene expression signature of lipodystrophic fat. Most genes and pathways that comprise this shared signature are new in the context of adipose tissue biology. Recurrent functional themes include up-regulation of genes regulating immunity, inflammation, and chemotaxis, and down-regulation of imprinted genes and secreted and membranal components of diverse extracellular signaling pathways [supporting information (SI) Table 1].

Discussion

We report here the generation and detailed analysis of a new mouse model of lipodystrophy, which confers the disease dom-

tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Pancreas sections were also stained with aldehyde fuchsin to detect insulin granules. Plasma analytes were determined in retroorbital blood collected from animals subject to 5–6 h of fasting. Glucose, triglycerides, free fatty acids, and cholesterol were measured in a Beckman (Fullerton, CA) Synchron CX5 Delta Clinical Chemistry Analyzer. Insulin was measured using a rat insulin RIA kit (LINCO, St. Charles, MO). For glucose tolerance tests, retroorbital blood was collected from 6 h-fasted animals, followed by i.p. injection of 2 g glucose per kilogram of body weight, collection of retroorbital blood samples 30, 60, 90, and 120 min thereafter, and determination of glucose concentrations in the Synchron Analyzer.

Adipogenesis of Embryonic Fibroblasts. To prepare fibroblasts carcasses of embryonic day 13.5 embryos were individually minced by 4–5 passages through an 18-gauge syringe needle. Cells were plated on 6-cm dishes, brought to complete confluence, and 2 days later were treated for 72 h with insulin (10 μ g/ml), 5 μ M dexamethazone, 0.2 mM isobutyl-methyl-xanthine, and 1 μ M rosiglitazone. Cultures were fed with fresh insulin-containing medium at 72-h intervals thereafter. Lipid content was assessed by Oil-red-O staining at the end of differentiation. In other experiments, RNA was extracted from cultures at different stages of differentiation and analyzed.

Affymetrix Microarrays and Statistical Analysis. RNA from epididymal fat pads of three 10-wk-old mutant males (*Pparg*^{deli/+} or *Sr*) and three litter-matched WT controls for each strain was treated with DNase I and probes were generated according to manufacturer's instructions (Affymetrix, Santa Clara, CA). Each sample hybridized to one MOE430 version 2.0 GeneChip array in a Fluidics Station 450 instrument (Affymetrix). Arrays were imaged with a GeneChip 3000 Laser Confocal Slide Scanner, were quantified using GeneChip Operating Software version 1.2 (Affymetrix), and expression values were summarized using the robust multichip average (RMA) function. *P* values were assigned using ANOVA methodology (25). False discovery rates (FDR) were assigned by computing *q* values from test statistics (26).

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