Partial lipodystrophy and insulin resistant diabetes in a patient with a homozygous nonsense mutation in CIDE C

Oscar Rubio-Cabezas1,14,15, Vishwajeet Puri2†, Incoronata Murano3, Vladimir Saudek4, Robert K. Semple4, Satya Dash4, Caroline S. S. Hyden4, William Bottomley5, Corinne Vigouroux6,7,8, Jocelyne Magré6,7, Philippa Raymond-Barker9, Peter R. Murgatroyd9, Anil Chawla2, Jeremy N. Skepper10, V. Krishna Chatterjee4, Sara Suliman11, LD Screening Consortium†, Ann-Marie Patch12, Anil K. Agarwal13, Abhimanyu Garg13, Inès Barroso5, Saverio Cinti3, Michael P. Czech2, Jesús Argente1,14,15, Stephen O'Rahilly4, David B. Savage4*

Keywords: lipodystrophy; insulin resistance; lipid droplet; CIDE C (Fsp27)

DOI 10.1002/emmm.200900037

Received April 14, 2009
Revised June 29, 2009
Accepted July 2, 2009

Lipodystrophic syndromes are characterized by adipose tissue deficiency. Although rare, they are of considerable interest as they, like obesity, typically lead to ectopic lipid accumulation, dyslipidaemia and insulin resistant diabetes. In this paper we describe a female patient with partial lipodystrophy (affecting limb, femorogluteal and subcutaneous abdominal fat), white adipocytes with multiloculated lipid droplets and insulin-resistant diabetes, who was found to be homozygous for a premature truncation mutation in the lipid droplet protein cell death-inducing DFFA-like effector C (CIDE C) (E186X). The truncation disrupts the highly conserved CIDE-C domain and the mutant protein is mistargeted and fails to increase the lipid droplet size in transfected cells. In mice, Cidec deficiency also reduces fat mass and induces the formation of white adipocytes with multilocular lipid droplets, but in contrast to our patient, Cidec null mice are protected against diet-induced obesity and insulin resistance. In addition to describing a novel autosomal recessive form of familial partial lipodystrophy, these observations also suggest that CIDEC is required for unilocular lipid droplet formation and optimal energy storage in human fat.

(1) Department of Endocrinology, Hospital Infantil Universitario Niño Jesús, Madrid, Spain.
(2) Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, USA.
(3) Institute of Normal Human Morphology, University of Ancona, Ancona, Italy.
(4) Metabolic Research Laboratories, Institute of Metabolic Science, University of Cambridge, Addenbrooke’s Hospital, Cambridge, UK.
(5) Metabolic Disease Group, The Wellcome Trust Sanger Institute, Hinxton, UK.
(6) UPMC Univ Paris O6, UMR_S938, Paris, France.
(7) INSERM, UMR_S938, Faculté de Médecine Pierre et Marie Curie, Site Saint-Antoine, Paris, France.
(8) AP-HP, Hôpital Tenon, Service de Biochimie et Hormonologie, Paris, France.
(9) Wellcome Trust Clinical Research Facility, Addenbrooke’s Hospital, Cambridge, UK.
(10) Multi-imaging Centre, Physiology Development & Neuroscience, University of Cambridge, Cambridge, UK.
(11) Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM), University of Oxford, Oxford, UK.
(12) Institute of Biomedical and Clinical Science (AM.P), Peninsula Medical School, Exeter, UK.
(13) Division of Nutrition and Metabolic Diseases, Department of Internal Medicine, Center for Human Nutrition, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA.
(14) Department of Pediatrics, Universidad Autónoma de Madrid; Madrid, Spain.
(15) CIBER Fisiopatología de la Obesidad y Nutrición (CIBERON), Instituto de Salud Carlos III, Madrid, Spain.

†These authors contributed equally to this work.
*Corresponding author: Tel: +44 1223 767923; Fax: +44 1223 330598; E-mail: dbs23@medschl.cam.ac.uk
INTRODUCTION

White adipose tissue (WAT) is essential for efficient energy (lipid) storage and release (Frayn, 2002). Although all eukaryotic cells can store surplus energy as lipid droplets, white adipocytes are uniquely adapted for the role. Several lipid droplet associated proteins are specifically expressed in white adipocytes, where they appear to be required for efficient lipid storage in and release from lipid droplets (Brasaemle et al., 2008; Traini & Jessup, 2009). Storing triglyceride and cholesterol esters in a single large droplet reduces the lipid droplet surface area available to lipolytic enzymes and optimizes the storage capacity. The importance of fat in human metabolism is highlighted by lipodystrophic syndromes, a heterogeneous cluster of disorders characterized by a lack of WAT (Garg, 2004). Lipodystrophy is distinct from leanness, a state in which ‘empty adipocytes’ can readily adapt to positive energy balance. Instead, in lipodystrophic subjects, positive energy balance leads to ectopic fat deposition in the liver and other organs, insulin resistance and diabetes (Savage et al., 2007).

Lipodystrophies result from either the failure of adipocyte development or premature destruction of adipocytes due to genetic or immunological mechanisms (Garg, 2004). Recent progress in understanding the genetic basis of several forms of familial lipodystrophy has facilitated improved clinical diagnostic workup in patients with lipodystrophy as well as providing novel insights into adipocyte biology. Biallelic loss of function mutations in BSCL2, AGPAT2 or CAV1 account for >90% of all cases of congenital generalized lipodystrophy, whereas heterozygous mutations in LMNA and PPARG account for >50% of all inherited cases of partial lipodystrophy (Garg & Agarwal, 2009). Rare homozygous and compound heterozygous mutations in LMNA and ZMPSTE24 have also been reported and, in one family with partial lipodystrophy, a heterozygous mutation in AKT2 was identified (George et al., 2004).

In this report, we describe a 14-year-old girl with a novel subtype of partial lipodystrophy and ‘ketosis-prone’ insulin resistant diabetes in association with a homozygous nonsense mutation in CIDEC.

CASE HISTORY

A 19-year-old Ecuadorian girl was referred with partial lipodystrophy and insulin resistant diabetes. She first presented at age 14 years with diabetic ketoacidosis (glucose 39.4 mmol/l; pH 7.25; bicarbonate 8.3 mmol/l; strongly positive plasma ketones). Clinically she was noted to have partial lipodystrophy with muscular lower limbs and prominent acanthosis nigricans (Fig 1A). This phenotype was reportedly present from early childhood. Her body mass index (BMI) was 20.8 kg/m² (BMI Standard deviation score (SDS) +0.52), blood pressure normal (103/64 mm Hg) and she had no signs of virilization or dysmorphic features. Menarche had occurred at age 12 years and menses were regular at presentation, but later became irregular. ICA (islet cell antibodies), anti-GAD (GAD, glutamic acid decarboxylase) and IA2 (protein tyrosine phosphatase-like protein) antibodies were all negative and C-peptide levels were elevated (1274 pmol/l (174–960)). Interestingly, her lipid profile was normal at presentation (total cholesterol 4.3 mmol/l, triglycerides 1.0 mmol/l) but worsening dyslipidaemia developed within 18 months, to the extent that she ultimately had pancreatitis secondary to hypertriglyceridaemia (triglycerides 20.2 mmol/l) and again presented with diabetic ketoacidosis (pH 6.77; strongly positive ketones). Despite high dose insulin therapy (1.6 IU/kg/day), her glycemic control was always poor (HbA1c 11.0–16.3%). She recently developed microalbuminuria and hypertension (blood pressure 153/96 mm Hg).

Magnetic resonance imaging (MRI) of her fat distribution confirmed the virtual absence of lower limb and femorogluteal fat pads and the preservation of visceral, neck and axillary fat (Fig 1B and Fig 1 of Supporting Information). Her total fat mass was 20% by DXA (dual energy X-ray absorptiometry), which is 1 SD below the age-matched mean. Plasma leptin (2.8 μg/l; BMI- and gender-matched reference range 2.4–24.4 μg/l) and adiponectin levels (1.7 mg/l; BMI- and gender-matched reference range 2.6–12.6 mg/l) were correspondingly low. She was also noted to have striking hepatomegaly and hepatic steatosis on MRI (Fig 1B).

RESULTS

Identification of a homozygous nonsense CIDE-C mutation

The proband was wild type for all coding exons and splice junctions of LMNA, PPARG, ZMPSTE24 and AKT2, so we went on to sequence additional candidate genes for lipodystrophy. We identified a homozygous transversion of guanine to thymine containing the homozygous segment was found to be 19.03-Mb (Woods et al., 2006). The mutation was confirmed in the proband by the homozygous genotype of the proband. The 3p26.3–3p24.3 interval was deleted by the SNPs rs163577–rs1707041.

Adipose tissue histology

Since CIDE-C (mouse orthologue is known as Fsp27) is a lipid droplet protein implicated in the formation of white adipocytes with unilocular lipid droplets in mice (Nishino et al, 2008; Toh

[57x325]mutations in
[57x347]for
[57x256]mutation in
[57x267]resistant diabetes in association with a homozygous nonsense
[57x279]subtype of partial lipodystrophy and ‘ketosis-prone’ insulin
[57x313]and, in one family with partial lipodystrophy, a heterozygous
[57x359]whereas heterozygous mutations in
[57x370]
[57x26]www.embomolmed.org
[57x26]EMBO Mol Med 1, 280–287

[57x382]of function mutations in
[57x393]providing novel insights into adipocyte biology. Biallelic loss
[57x405]nostic workup in patients with lipodystrophy as well as
[57x416]familial lipodystrophy has facilitated improved clinical diag-
[57x428]progress in understanding the genetic basis of several forms of
[57x439]genetic or immunological mechanisms (Garg, 2004). Recent
[57x473]2007).

Childhood. Her body mass index (BMI) was 20.8 kg/m² (BMI
[57x508]was 20% by DXA (dual energy X-ray absorptiometry), which is
[57x519]was 20% by DXA (dual energy X-ray absorptiometry), which is
[57x531]fat pads and the preservation of visceral, neck and axilliary fat
[57x542]confirmed the virtual absence of lower limb and femorogluteal
[57x565]microalbuminuria and hypertension (blood pressure 153/

[57x611]ketoacidosis (pH 6.77; strongly positive ketones). Despite high
dose insulin therapy (1.6 IU/kg/day), her glycemic control was
always poor (HbA1c 11.0–16.3%). She recently developed
microalbuminuria and hypertension (blood pressure 153/96 mm Hg).

Magnetic resonance imaging (MRI) of her fat distribution
confirmed the virtual absence of lower limb and femorogluteal
fat pads and the preservation of visceral, neck and axilliary fat
(Fig 1B and Fig 1 of Supporting Information). Her total fat mass
was 20% by DXA (dual energy X-ray absorptiometry), which is
1 SD below the age-matched mean. Plasma leptin (2.8 μg/l; BMI-
and gender-matched reference range 2.4–24.4 μg/l) and adipono-
ectin levels (1.7 mg/l; BMI- and gender-matched reference
range 2.6–12.6 mg/l) were correspondingly low. She was also
noted to have striking hepatomegaly and hepatic steatosis on
MRI (Fig 1B).

RESULTS

Identification of a homozygous nonsense CIDE-C mutation

The proband was wild type for all coding exons and splice
junctions of LMNA, PPARG, ZMPSTE24 and AKT2, so we went
on to sequence additional candidate genes for lipodystrophy.
We identified a homozygous transversion of guanine to thymine
at CDNA nucleotide position 556 in exon 6 of CIDE-C, resulting
in the substitution of a premature stop codon (TAA) for a
glutamine codon (GAA) at codon 186 (Fig 1B and Fig 1 of
Supporting Information). The mutation is predicted to truncate
the protein at amino acid 186, resulting in loss of a significant
portion of the CIDE-C domain of CIDE-C (Fig 1D). It was absent
in 120 ethnically matched control alleles. The proband’s
unaffected mother was heterozygous for the CIDE-C E186X variant. Her BMI, fat distribution and biochemical parameters were all entirely
normal (Fig 1E). Two maternal half brothers were wild type for
this variant and manifested normal fat distribution and biochem-
istry. The proband’s father was not available for genetic testing but
single nucleotide polymorphism (SNP) genotyping analysis of the
proband revealed a total genomic homozygosity value of 13.5%
which is consistent with parental consanguinity (A-M Patch,
unpublished observations; Woods et al, 2006). The mutation
containing the homozygous segment was found to be 19.03-Mb
long, spanning 3p26.3–3p24.3 delimited by the SNPs rs163577–
rs1707041.

Adipose tissue histology

Since CIDE-C (mouse orthologue is known as Fsp27) is a lipid
droplet protein implicated in the formation of white adipocytes
with unilocular lipid droplets in mice (Nishino et al, 2008; Toh
et al, 2008), the proband went on to have a subcutaneous fat biopsy. The most striking finding in the tissue sample was the presence of many adipocytes with multiple small lipid droplets, each of which was strongly immunoreactive to perilipin (so called ‘multilocular adipocytes’), rather than the normal single large droplet (Fig 2A). The number of lipid droplets per cell varied inversely with cell size (Fig 2B). Unilocular adipocytes were similar in size to adipocytes from a lean control (Fig 2 of Supporting Information). Immunohistochemistry for UCP1 (uncoupling protein 1) was negative (data not shown), suggesting that these were white-, not brown adipocytes, but intense focal cytochrome c immunoreactivity in some cells with multilocular lipid droplets suggests that mitochondrial mass is increased in at least some of the proband’s adipocytes (Fig 2C). Electron microscopy (EM) confirmed the multilocular nature of lipid droplets in many white adipocytes (Fig 2D) and the apparent focal increase in mitochondrial density (Fig 2D). There was no evidence of excess crown-like structures or inflammation in the adipose tissue.

Although the ‘multilocular adipocytes’ seen in this patient are not typical brown adipocytes, we wondered if these cellular changes might alter the resting metabolic rate (RMR). The proband’s RMR was significantly increased when compared to healthy controls. The elevation in RMR remained after correction for lean body mass (Fig 3A of Supporting Information) and for fat and lean mass (Fig 3B of Supporting Information). Thyroid function tests and plasma metanephrine concentrations were normal (data not shown).

Cellular characterization of the E186X CIDEC mutant
In order to examine the function of the mutant CIDEC protein, we electroporated COS cells and 3T3L1 pre-adipocytes with N-terminal green fluorescent protein-tagged (GFP-tagged) human CIDEC vectors. Although lipid droplet size was significantly increased in COS cells over expressing wild type CIDEC compared to untransfected cells (Fig 3A), lipid droplet size was not altered in cells over expressing the mutant protein at a similar level (Fig 3A). Similar effects were observed in 3T3L1 pre-adipocytes (Fig 3B). Formal morphometric analysis confirmed these visual observations in both cell types (Fig 3C) and suggested that the mutant protein is biologically inert, at least in terms of the protein’s actions on lipid droplet size in cultured cells. Overlay of the GFP and oil-red-O stained images shows some co-localization of wild type CIDEC and lipid droplets whereas this is not seen in cells expressing the truncated CIDEC protein (Fig 3A,B). The fact that most of the WT CIDEC is not

---

**Figure 1. Identification of a homozygous premature stop E186X CIDEC mutation in a patient with acanthosis nigricans and partial lipodystrophy.**

A. Photograph demonstrating axillary acanthosis nigricans in the proband.

B. T1 weighted magnetic resonance image of the proband indicating the paucity of leg-, forearm-, femorogluteal- and subcutaneous abdominal fat. Axillary fat and neck fat are preserved. The liver is significantly enlarged due to severe steatosis. She also manifests prominent muscle bulk.

C. Wild type CIDEC, E186X CIDEC homozygous (Homo; proband) and heterozygous (Het; proband’s mother) mutant sequence traces. Homozygous transversion of guanine to thymine at nucleotide position 556 in exon 6 of CIDEC, results in the substitution of a premature stop codon (TAA) for glutamine (GAA) at codon 186.

D. Schematic representation of CIDEC showing the position of the E186X premature stop mutation (red line), resulting in loss of a significant portion of the CIDE-C domain (grey) of CIDEC. The CIDE-N domain is highlighted in black and amino acid numbers are indicated below.

E. Proband’s family pedigree (squares represent male and circles female family members). Below each symbol, age (years) is given, followed by the BMI (kg/m²), fasting glucose (mmol/l), insulin (pmol/l), HOMA (homeostasis model assessment value; %), triglycerides (mmol/l), free fatty acids (µmol/l), leptin (µg/l) and genotype, with N denoting the normal (wild type) allele and M the mutant allele.

BMI for children is given as BMI SDS. Filled symbol represents the affected proband and hatched symbol her heterozygous mother.
localized around lipid droplets in these cells could be due to the need for other differentiation-dependent proteins present in adipocytes to facilitate CIDE localization to lipid droplets. Thus we also assessed localization of the wild type and E186X CIDE mutant in transfected adipocytes (six days after initiating differentiation). Whereas most of the wild type CIDE is localized around lipid droplets in these transfected adipocytes, the CIDE truncated mutant is not (Fig 4), suggesting that the C-terminal part of CIDE is required for lipid droplet localization.

**DISCUSSION**

In a patient with unexplained partial lipodystrophy, we identified a homozygous premature stop mutation in CIDE. CIDE/Fsp27 is a lipid droplet protein most highly expressed in adipocytes where its expression is induced during adipocyte differentiation (Keller et al, 2008; Puri et al, 2007). Fsp27 knockdown in 3T3L1 pre-adipocytes reduces triglyceride accumulation and lipid droplet size, and increases lipid droplet number and lipolysis (Keller et al, 2008; Nishino et al, 2008). In keeping with these data, Fsp27 knockout (KO) mice manifest reduced adipose tissue mass and white adipocytes with multilocular lipid droplets (Nishino et al, 2008; Toh et al, 2008). CIDE is expressed in human WAT where its mRNA level is inversely correlated with fat mass, reduced by a very low calorie diet (Magnusson et al, 2008), and lower in adipose tissue from obese insulin resistant compared to equally obese insulin sensitive subjects (Puri et al, 2008). The latter observation is particularly interesting as it suggests that reduced CIDE expression may contribute to the pathogenesis of obesity-induced insulin resistance.

The CIDE E186X mutation disrupts the highly conserved CIDE-C domain which is known to be required for the protein’s function (Chen et al, 2000; Keller et al, 2008). Expressing the N-terminal half of Fsp27 in fibroblasts had no effect on lipid droplet formation, whereas expression of the C-terminal CIDE-C domain was sufficient to stimulate lipid accumulation (Keller et al, 2008). The homologous CIDE-C domain of Cideb is required for phospholipid membrane targeting and appears to be equally critical for its biological activity (Ye et al, 2009). When expressed in COS cells or 3T3L1 pre-adipocytes, the mutant CIDE protein is mistargeted and has similar effects on lipid droplet morphology to those of an empty vector, whereas expression of wild type CIDE significantly enhanced the volume of cellular lipid droplets. The truncated CIDE protein is also mistargeted in differentiated 3T3L1 adipocytes where the bulk of wild type CIDE localizes around lipid droplets. The most striking finding in our patient was the presence of many white adipocytes with multilocular lipid droplets and focal increases in mitochondrial mass. These observations are consistent with what was seen in Fsp27 knockdown cells and in Fsp27 null mice (Nishino et al, 2008; Toh et al, 2008) but in contrast to the KO mouse phenotype, these changes were associated with an adverse lipodystrophic, rather than a healthy lean, phenotype. The presence of severe dyslipidaemia, fatty liver, low leptin and adiponectin levels, coupled with insulin resistance and diabetes in the CIDE E186X proband is typical of human lipodystrophy (Garg, 2004). Discrepancies between mouse and human phenotypes have been reported previously: PPARG P467L and other ligand binding domain variants consistently cause lipodystrophy and severe insulin resistance in humans (Semple et al, 2006), whereas mice with the equivalent Pparg P465L mutation are neither lipodystrophic nor...
insulin resistant (Gray et al, 2006). It is currently too early to say if this discrepancy indicates the existence of species-specific differences in the global metabolic response to perturbations of adipocyte biology.

In Fsp27 KO mice, all white adipocytes appeared to have multilocular lipid droplets (Nishino et al, 2008; Toh et al, 2008), whereas we observed adipocytes with unilocular and paucilocalar lipid droplets in addition to the multilocular cells (Fig 2A). Until more is known about the function of CIDEC, we can only speculate about the molecular origins of this finding. Although our transfection studies suggest that the truncated protein fails to increase lipid droplet size, it remains possible that it does retain some \textit{in vivo} activity (for instance, the CIDE-N domain remains intact). The progressive increase in the size of adipocytes with fewer and ultimately a single lipid droplet compared to multilocular cells (Fig 2B) also suggests that lipid droplets may eventually fuse to generate a single large droplet despite ‘CIDEC deficiency’. CIDEA, another member of the CIDE family of proteins, is predominantly expressed in brown, rather than white, adipose tissue in mice, but is expressed in white adipocytes in humans (Puri et al, 2008). It is therefore also possible that CIDEA may compensate, at least in part, for the loss of CIDEC function. We have sequenced the coding regions of CIDEA in our patient and her family. She was heterozygous for an arginine to tryptophan substitution at codon 5 (R5W) (data not shown). This variant was also present in one of her half-brothers, who was phenotypically normal, and in 16.7% of 108 South American control alleles. It is located in an N-terminal lead sequence before the CIDE-N domain and hence is not expected to alter the protein structure. These observations suggest that the CIDEA variant is a common polymorphism and is unlikely to be pathogenic but we cannot exclude the possibility that it might contribute to the proband’s extreme phenotype.

The perception that converting white adipocytes into thermogenic brown adipocytes could cause weight loss and improve insulin sensitivity has prompted successful attempts to do this in rodents. These efforts included both genetic (Tsukiyama-Kohara et al, 2001) and pharmacological approaches such as chronic treatment with a selective \( \beta \)-adrenergic agonist (Himms-Hagen et al, 2000). In all cases, UCP1 expression and mitochondrial mass were increased in multilocular adipocytes. Although the limited amount of available tissue meant that we could not formally assess mitochondrial mass or function in WAT from the \textit{CIDEC} E186X proband, cytochrome \( c \) immunoreactivity and electron micrographs suggested that mitochondrial mass was increased. The absence of UCP1 immunostaining suggests that these cells are not brown adipocytes. Nevertheless, are these multilocular adipocytes likely to account for the increased RMR observed in our patient? It is currently not possible to accurately measure the
tissue specific contributions to the total energy expenditure in humans, but the mass specific contribution of fat to energy expenditure is roughly 17 kJ/kg/day, about one sixth of that of lean mass (Nelson et al, 1992). To account for a 1.3 kJ/min elevation in resting energy expenditure relative to healthy volunteers, fat associated energy expenditure would need to be double that seen in healthy fat metabolism. Changes in oxygen consumption of this magnitude were recorded in adipocytes of volunteers, fat associated energy expenditure would need to be double that seen in healthy fat metabolism.

In summary, we report a novel autosomal recessive cause of partial lipodystrophy. The clinical phenotype is similar to that caused by mutations in LMNA, PPARG and AKT2, all of which manifest striking lower limb and femorogluteal lipodystrophy, fatty liver, dyslipidaemia and insulin-resistance. Together with observations made in cultured cells and Fsp27 KO mice, our observations also suggest that CIDEC is required for optimal energy storage in ‘unilocular adipocytes’ in humans, although we recognize that this statement is tempered by the fact that it is currently based on observations in a single small kindred.

METHODS

Genetic and phenotypic studies

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the UK National Health Service Research Ethics Committee. Each participant, or a parent in the case of minors, provided written informed consent; minors provided oral consent.

Genomic DNA was isolated from peripheral-blood leukocytes. After excluding mutations in the coding regions and splice junctions of LMNA, PPARG, ZMPSTE24 and AKT2, the coding regions and splice junctions of CIDEC were amplified by PCR and sequenced (primer sequences available upon request). Insulin sensitivity was calculated using HOMA %S (homeostasis model assessment) (Levy et al, 1998). Body composition was measured by whole-body DXA and fat distribution was assessed using a 1.5 T MR scanner to acquire T1-weighted water suppressed images. RMR was measured using a ventilated canopy indirect calorimeter after an overnight fasting. Subcutaneous fat was studied using light microscopy, immunohistochemistry, and EM (see Supporting Information for details).

Cellular functional analysis

3T3-L1 pre-adipocytes and COS cells were cultured and differentiated in standard conditions (Puri et al, 2008). For cells incubated with fatty acids, 20 µM oleic acid/BSA mixture was added to the medium 8 h after transfection. CIDEC plasmid DNA was procured from Open Biosystems. For full length CIDEC, PCR was performed by using a 5'-linker with a BglII restriction site and a 3'-linker with an HindIII site. Following a PCR product, the fragment was cloned into pEGFP-C1 vector (Clontech, USA). GFP–CIDEC cDNA (5 µg) was transfected into COS cells using the Lipofectamine Plus Reagent (GIBCO Life Technologies, Rockville, Maryland). Pre-adipocytes and adipocytes were electroporated with 6 µg cDNA (200,000 cells/200 µl PBS) in a 0.4 cm cuvette at 180 V and 950 µF with a time constant of 25 ms on a Bio-Rad Gene Pulser II system. After transfection, the cells were cultured for 24–48 h before fixing and staining with oil red. Oil-red-O...
The paper explained

PROBLEM:
Lipodystrophy is a rare disease characterized by a partial or complete lack of adipose tissue. Paradoxically, both ‘too much’ (obesity) and ‘too little’ (lipodystrophy) fat lead to adipose tissue dysfunction, ectopic lipid accumulation, insulin resistance and diabetes.

Within the last decade, investigators have identified mutations in seven different genes in patients with lipodystrophy. In this study, we focused on a novel subtype of partial lipodystrophy.

RESULTS:
We report the identification of a homozygous nonsense mutation in CIDEK in a patient with partial lipodystrophy, fatty liver, severe insulin resistance, dyslipidaemia and diabetes. Histological studies of residual adipose tissue from the patient revealed white adipocytes with multiloculated lipid droplets and excess mitochondria. Functional studies of the mutant protein indicated that it fails to increase the lipid droplet size and, contrary to the wild type protein, it does not localize around lipid droplets in transfected cells.

IMPACT:
The results add the CIDEK gene to the list of genes whose mutation is associated with lipodystrophy. CIDEK is a recently identified lipid droplet protein whose function remains incompletely understood. CIDEK (Fsp27) null mice manifest low fat mass and multilocular adipocytes with excess mitochondria, but in contrast to our patient, these mice are insulin sensitive. Together, these studies suggest that CIDEK is required for unilocular lipid droplet formation and optimal lipid storage in white adipose tissue. The differences between the human and mouse phenotypes raise intriguing questions about interspecies differences in adipose tissue metabolism.

Author contributions
Oscar Rubio Cabezas, Jesús Argente and David Savage conducted the clinical investigations. Vishwajeet Puri, aided by Anil Chawla, performed the functional studies which he planned together with Michael Czech, Stephen O’Rahilly and David Savage. Incoronata Murano, Jeremy Skepper and Saverio Cinti performed the histological studies. Vladimir Saudek provided bioinformatic analyses on all the mutations identified. Oscar Rubio Cabezas, Robert Semple, Satya Dash, Caroline Hyden, William Bottomley, Ann-Marie Patch, Ines Barroso, Stephen O’Rahilly and David Savage contributed to the genetic studies. Philippa Raymond-Barker, Peter Murgatroyd and David Savage analysed the metabolic rate data and provided reference data from a control sample. Robert Semple, Corinne Vigouroux, Jocelyne Magré, V. Krishna Chatterjee, Sara Sullman, LD Screening Consortium, Anil Agarwal, Abhimanyu Garg, Stephen O’Rahilly and David Savage contributed DNA samples from patients with unexplained lipodystrophy. Oscar Rubio Cabezas, Vishwajeet Puri, Jesús Argente, Stephen O’Rahilly and David Savage wrote the paper, which was then reviewed by all the authors.

Acknowledgements
We thank the participants. We thank Margaret Blount for assistance with histological analysis. O.R.C. is supported by an ‘Ayuda para contratos post-Formación Sanitaria Especializada’ from the ‘Instituto de Salud Carlos III’ (FIS CM06/00013), Spain. This work was also supported by grants from the Wellcome Trust (R.K.S., V.K.K.C., S.O’R., D.B.S., W.B., I.B. (WT Grant 077016/Z/05/Z), CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN) from the ‘Instituto de Salud Carlos III’ and Fundación Endocrinología y Nutrición, Madrid, Spain (J.A.), GlaxoSmithKline (D.B.S.), the U.K. NIHR Cambridge Biomedical Research Centre, National Institutes of Health grants DK30898, DK60837 (to M.P.C.) and DK54387 (to A.G.), the Biomedical Imaging Core Facility of the University of Massachusetts Diabetes and Endocrinology Center (National Institutes of Health grant DK32520) (M.P.C.), Diabetes UK (S.S.), the Italian Ministry of University (FIRB RBIN047PZY_000 Internazionalizzazione, to S.C.) and Cofin. PRIN bando 2007 (S.C.), Aide aux Jeunes Diabétiques (AJD), Association de Langue Française pour l’Etude du Diabète et des Maladies Métaboliques (ALFEDIAM) (J.M.) and from the French National Institute for Health and Medical Research (INSERM) (J.M., C.V.).
Supporting information is available at EMBO Molecular Medicine online.

The authors declare that they have no conflict of interest.

For more information

Online Mendelian Inheritance in Man:
CIDEC:
http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=612120

David Savage Laboratory:
http://www.mrl.ims.cam.ac.uk/staff/PI/Savage/index.php

References

Brasaemle DL, Subramanian V, Garcia A, Marcinkiewicz A, Rothenberg A, (2009) Perilipin A and the control of triacylglycerol metabolism. Mol Cell Biochem 326: 15-22
Chen Z, Guo K, Toh SY, Zhou Z, Li P (2000) Mitochondria localization and dimerization are required for CIDEB to induce apoptosis. J Biol Chem 275: 22619-22622
Frayn KN (2002) Adipose tissue as a buffer for daily lipid flux. Diabetologia 45: 1201-1210
Garg A (2004) Acquired and inherited lipodystrophies. N Engl J Med 350: 1220-1234
Garg A, Agarwal AK (2009) Lipodystrophies: disorders of adipose tissue biology. Biochim Biophys Acta 1791: 507-513
George S, Rochford JJ, Wolfrum C, Gray SL, Schinner S, Wilson JC, Soos MA, Murgatroyd PR, Williams RM, Acerini CL, et al (2004) A family with severe insulin resistance and diabetes due to a mutation in AKT2. Science 304: 1325-1328
Gray SL, Dalla Nora E, Backlund EC, Manieri M, Virtue S, Noland RC, O'Rahilly S, (2006) FSP27 contributes to efficient energy storage in murine white adipocytes by promoting the formation of unilocular lipid droplets. J Clin Invest 118: 2808-2821
Levy JC, Matthews DR, Hermans MP (1998) Correct homoeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 21: 2191-2192
Magnusson B, Gummesson A, Glad CA, Goedecke JH, Jernas M, Lystig TC, Carlsson B, Fagerberg B, Carlsson LM, Svensson PA (2008) Cell death-inducing DFF45-like effector C is reduced by caloric restriction and regulates adipocyte lipid metabolism. Metabolism 57: 1307-1313
Nelson KM, Weinsier RL, Long CL, Schutz Y (1992) Prediction of resting energy expenditure from fat-free mass and fat mass. Am J Clin Nutr 56: 848-856
Nishino N, Tamori Y, Tateya S, Kawaguchi T, Shibakusa T, Mizonoya W, Inoue K, Kitazawa R, Kitazawa S, Matsuki Y, et al (2008) FSP27 contributes to efficient energy storage in murine white adipocytes by promoting the formation of unilocular lipid droplets. J Clin Invest 118: 2808-2821
Puri V, Czech MP (2008) Lipid droplets: FSP27 knockout enhances their sizzle. J Clin Invest 118: 2693-2696
Puri V, Konda S, Ranjit S, Anaudi M, Chawla A, Chouinard M, Chakladar A, Czech MP (2007) Fat-specific protein 27, a novel lipid droplet protein that enhances triglyceride storage. J Biol Chem 282: 34213-34218
Puri V, Ranjit S, Konda S, Nicoloro SM, Straubhaar J, Chawla A, Chouinard M, Lin C, Burkart A, Corvera S, et al (2008) CIDEB is associated with lipid droplets and insulin sensitivity in humans. Proc Natl Acad Sci USA 105: 7833-7838
Savage DB, Petersen KF, Shulman GI (2007) Disordered lipid metabolism and the pathogenesis of insulin resistance. Physiol Rev 87: 507-520
Semple RK, Chatterjee VK, O'Rahilly S (2006) PPAR gamma and human metabolic disease. J Clin Invest 116: 581-589
Toh SY, Gong J, Du G, Li JZ, Yang S, Ye J, Yao H, Zhang Y, Yue B, Li Q, et al (2008) Up-regulation of mitochondrial activity and acquisition of brown adipose tissue-like property in the white adipose tissue of fsp27 deficient mice. PLoS ONE 3: e2890
Traini M, Jessup W (2009) Lipid droplets and adipose metabolism: a novel role for FSP27/CIDEC. Curr Opin Lipidol 20: 147-149
Tsukiyama-Kohara K, Poulin F, Kohara M, DeMaria CT, Cheng A, Wu Z, Gingras AC, Katsumi A, Elchebly M, Spiegelman BM, et al (2001) Adipose tissue reduction in mice lacking the translational inhibitor 4E-BP1. Nat Med 7: 1128-1132
Woods CG, Cox J, Springell K, Hampshire DJ, Mohamed MD, McKibbin M, Stern R, Raymond FL, Sandford R, Malik Sharif S, et al (2006) Quantification of homozygosity in consanguineous individuals with autosomal recessive disease. Am J Hum Genet 78: 889-896
Ye J, Li JZ, Liu Y, Li X, Yang T, Ma X, Li Q, Yao Z, Li P (2009) CIDEB, an ER- and lipid droplet-associated protein, mediates VLDL lipidation and maturation by interacting with apolipoprotein B. Cell Metab 9: 177-190