SUPPLEMENTARY DATA

SUPPLEMENTARY METHODS

Genetic analysis

CIDEC sequences were compared to the published *CIDEC* template (GenBank accession number, NM_022094) and published polymorphisms. Following identification of the homozygous c.556G>T mutation in *CIDEC*, the exon 6 PCR was repeated with an independent set of flanking primers to formally exclude the possibility of a primer binding site polymorphism in the paternal allele. SNP genotyping was carried out on the Affymetrix 5.0 mapping chip by ALMAC Diagnostics Carigavon, Northern Ireland. Processing of genomic DNA was performed as per the Affymetrix protocol and the SNP call rate was 99.5%. Genomic homozygous segments were identified using Perl scripts and were defined as stretches of consecutive homozygous SNPs that exceeded 3 Mb allowing for a maximum of 2 heterozygous SNPs per 100 calls. Total genomic homozygosity was calculated as the sum of homozygous bases, within segments that met the thresholds, expressed as a percentage of the genome covered.

CIDEC was sequenced in 168 additional probands with unexplained partial lipodystrophy referred to members of the Lipodystrophy Screening Consortium for genetic testing.

Resting metabolic rate (RMR)

Participants were requested to refrain from strenuous exercise 24 hours prior to study and to avoid caffeinated drinks and nicotine during their research stay. Baseline measurements of composition of room air were made before and after each measurement. During RMR measurement, participants were asked to remain awake, but relaxed and motionless.

Multiple linear regression analysis was performed to relate measured RMR (kJ/min) to fat and lean mass (kg) in a group of 35 healthy volunteers (females = 21, males = 14)[1, 2]. Residuals between measured RMR and RMR predicted by the regression model were

derived. This regression model was used to estimate the RMR of the proband. The difference between this and her measured RMR was examined in the context of the residuals of the healthy volunteers.

Histological analysis

For light microscopy and immunohistochemistry (IHC), tissue was fixed overnight at 4°C in 4% paraformaldehyde. It was then washed in phosphate buffer (0.1M; PH 7.4), dehydrated in ethanol, paraffin embedded and cut into 3µm thick sections. For IHC, 3µm dewaxed serial sections were incubated with anti-perilipin (PREK, kindly provided by AS Greenberg, Boston, MA), anti-UCP1 (kindly provided by Dr. D. Ricquier, Paris, France) and anti-cytochrome C (Santa Cruz, CA, USA) primary antibodies according to the ABC method[3]. We used 3% hydrogen peroxide to inactivate endogenous peroxidase followed by normal goat or rabbit serum to reduce non-specific staining. Consecutive serial sections were incubated overnight (4°C) with anti-perilipin (1:50), and anti-UCP1 (1:4500) and anti-cytochrome C (1:50) primary antibodies. Biotinylated HRP (horse radish peroxidase)-conjugated secondary antibodies were goat anti-rabbit IgG (perilipin and cytochrome C) and rabbit anti-sheep IgG (UCP1; Vector Laboratories; Burlingame, CA, USA). Histochemical reactions were performed using Vector's Vectastain ABC Kit and Sigma Fast 3,3'-diaminobenzidine as substrate (Sigma, St Louis, MO, USA). Sections were counterstained with haematoxylin. Negative control was obtained by omitting the primary antibody. Adipocytes were identified as unilocular, paucilocular (a large vacuole surrounded by ≤5 small lipid droplets), or multilocular (>5 small lipid droplets). Unilocular, paucilocular and multilocular adipocyte area was measured on H&E (haematoxylin and eosin) sections, assuming a spherical shape of these cells. Adipocyte profiles from well-preserved areas were drawn using a digital image analysis system and their surface area was measured by LM (light miscroscopy, x20) using the Nikon LUCIA IMAGE program (Laboratory Imaging, Praha, Czech Republic). Electron microscopy was performed according to standard protocols.

SUPPLEMENTARY RESULTS

Identification of additional novel *CIDEC* variants.

168 probands with partial lipodystrophy of unknown cause were screened for *CIDEC* mutations. All of these were wild type for *LMNA, PPARG* and *ZMPSTE24*. Three novel heterozygous nonsynonomous variants were identified – two of these are located in the CIDE-N domain, the structure of which can be predicted by homology[4], and the other is located in the hinge region between the CIDE-N and CIDE-C domains.

1) Cytosine 1930 Thymine (T49M). A heterozygous mutation which changes threonine at position 49 to methionine was identified in 6 patients and 9 of 90 ethnically matched healthy controls. The mutation lies at the beginning of the CIDE-N domain within the structured core. It packs against several other amino acids. It does not affect the dimerisation interface. The position is occupied by threonine in almost all therian mammals (serine in two cases). All other vertebrates have asparagine at this position. The mutation may influence the conformation of the domain but no drastic change is expected.

2) Thymine 1965 Cytosine (Y61H). A heterozygous mutation which changes tyrosine at position 61 to histidine was identified in 4 patients and 1 of 90 ethnically matched healthy controls. The mutation is in a variable position in a stretch of conserved residues. Only apes have tyrosine at this position. Histidine is common among mammals including a primate Otolemur garnettii. Tyrosine 61 lies in a surface loop between the second beta-sheet and first helix and is exposed to solvent. The mutation is unlikely to destabilise the CIDE-N structure and is outside the dimerisation surface.

3) Alanine 135 Threonine (A135T). A heterozygous mutation which changes alanine at position 135 to threonine was identified in 1 African-American patient. Alanine 135 is a non-conserved residue in a highly variable region of CIDEC between the CIDE-N and CIDE-C domains. The mutation was not present in two affected first degree relatives of the proband.

References

1. Nelson KM, Weinsier RL, Long CL, Schutz Y. Prediction of resting energy expenditure from fat-free mass and fat mass. Am J Clin Nutr 1992;56:848-56.

2. Nielsen S, Hensrud DD, Romanski S, Levine JA, Burguera B, Jensen MD. Body composition and resting energy expenditure in humans: role of fat, fat-free mass and extracellular fluid. Int J Obes Relat Metab Disord 2000;24:1153-7.

3. Hsu SM, Raine L. Protein A, avidin, and biotin in immunohistochemistry. J Histochem Cytochem 1981;29:1349-53.

4. Lugovskoy AA, Zhou P, Chou JJ, McCarty JS, Li P, Wagner G. Solution structure of the CIDE-N domain of CIDE-B and a model for CIDE-N/CIDE-N interactions in the DNA fragmentation pathway of apoptosis. Cell 1999;99:747-55.

SUPPLEMENTARY FIGURES

Supplementary figure 1. T1-weighted magnetic resonance images showing fat distribution in the E186X CIDEC proband. Transverse images are shown at the following levels: neck; abdomen (umbilicus); gluteal and calf.

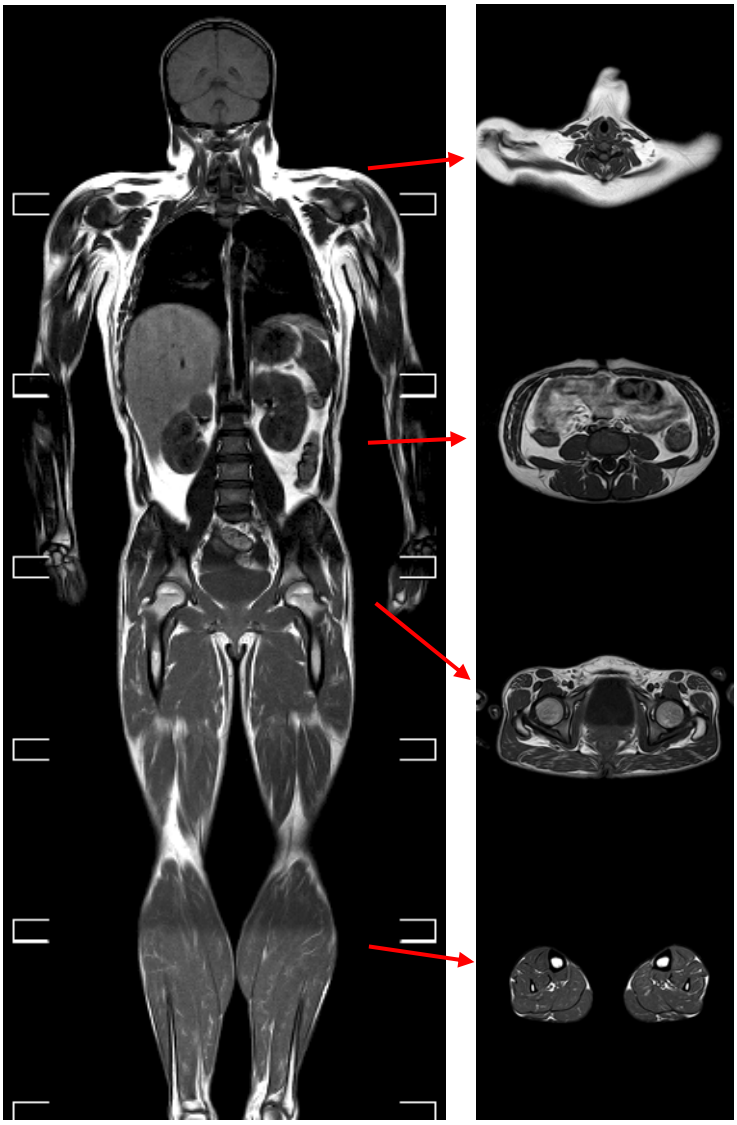
Supplementary figure 2. Morphometric analysis of unilocular adipocyte cell size in the E186X CIDEC proband (E186X) and a lean (BMI 25.1 kg/m^2), healthy control (Control). Cell size measurements were based on analysis of 21 cells in each case. Data is expessed as mean ± SE.

Supplementary figure 3. Energy expenditure in the homozygous E186X CIDEC proband. (A) Resting metabolic rate (RMR) versus lean body mass (LBM) plot for the E186X *CIDEC* proband (red triangle), healthy control females (n=21, mean (range): for age 39.9 years (24.2–61.4), BMI 23.5 kg/m^2 (18.1-30.9), green squares) and males (n=14, mean (range): age 37.2 years (17.4-53.4), BMI 24.7 kg/m^2 (18.7-29.4), blue circles). The green and blue lines represent linear regressions for female and male control data respectively (r= 0.74 and 0.86).

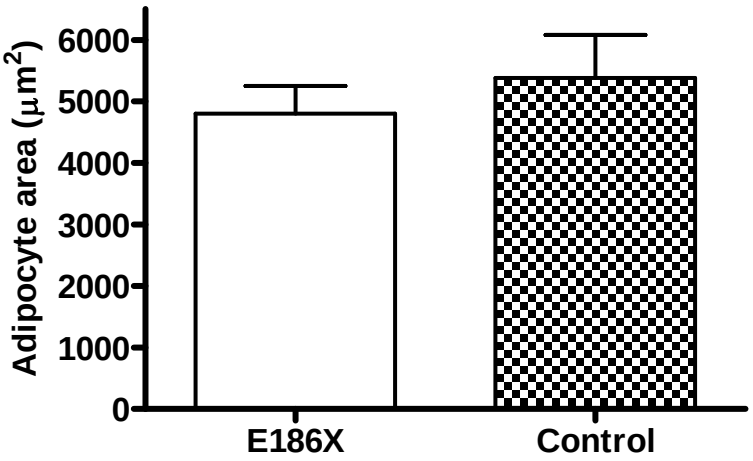
(B) Multiple regression analysis was then performed using lean body mass and fat mass as independent variables, with measured RMR being the dependent variable. Data points represent residuals between estimates from this regression and the measured RMR. Dotted lines represent the mean ± 2SDs.

Supplementary figure 4. Expression of GFP vector alone has no effect on lipid distribution and/or accumulation in 3T3-L1 preadipocytes (or in COS cells (data not shown)). The cells were transfected for 24 hr with GFP vector alone and fed with OA/BSA. Bar length 10 μM.

Supplementary figure 1

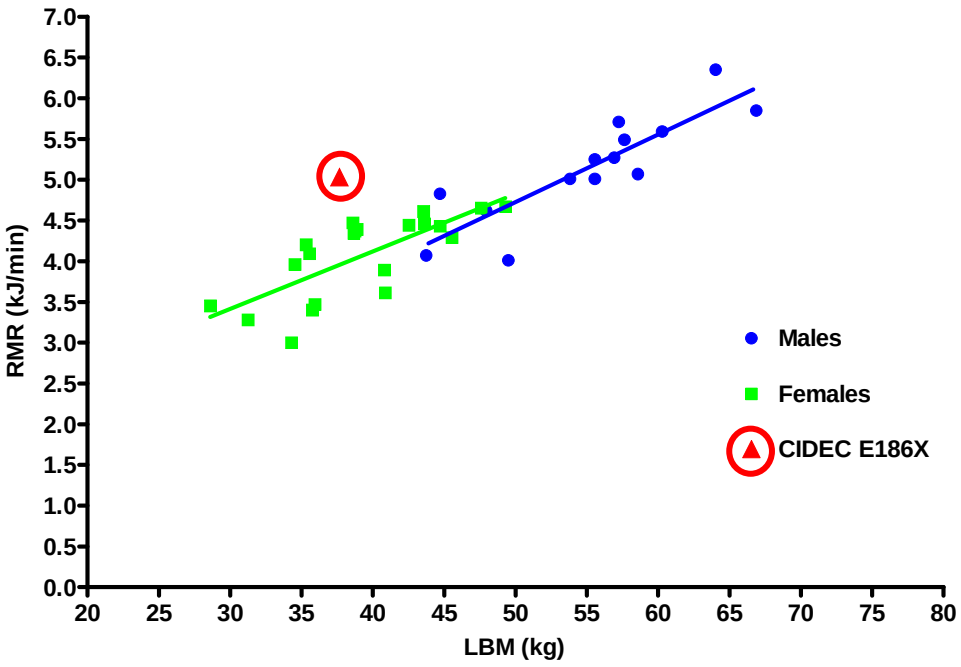


Supplementary figure 2



Supplementary figure 3

A



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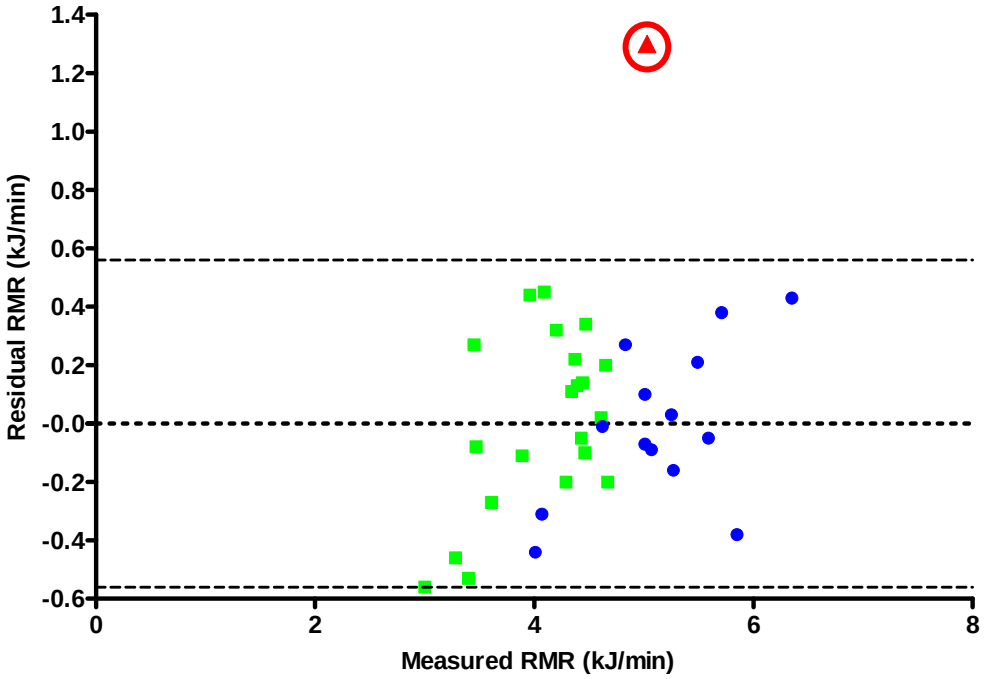

Supplementary figure 4