



Homozygous deletion of an 80kb region comprising part of DNAJC6 and LEPR genes on chromosome 1P31.3 is associated with early onset obesity, mental retardation and epilepsy.

Virginie Vauthier, Sylvie Jaillard, Hubert Journal, Christèle Dubourg, Ralf Jockers, Julie Dam

► To cite this version:

Virginie Vauthier, Sylvie Jaillard, Hubert Journal, Christèle Dubourg, Ralf Jockers, et al.. Homozygous deletion of an 80kb region comprising part of DNAJC6 and LEPR genes on chromosome 1P31.3 is associated with early onset obesity, mental retardation and epilepsy.: Homozygous deletion of the DNAJ6C and the LEPR gene. *Molecular Genetics and Metabolism*, Elsevier, 2012, 106 (3), pp.345-50. <10.1016/j.ymgme.2012.04.026>. <inserm-00703693>

HAL Id: inserm-00703693

<http://www.hal.inserm.fr/inserm-00703693>

Submitted on 6 Jun 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

TITLE

HOMOZYGOUS DELETION OF A 80 KB REGION COMPRISING THE *DNAJ6C* GENE AND PART OF THE *LEPR* GENE ON CHROMOSOME 1P31.3 IS ASSOCIATED WITH EARLY ONSET OBESITY, MENTAL RETARDATION AND EPILEPSY

Correspondence should be addressed to: Ralf Jockers, Institut Cochin, 22 rue Méchain, 75014 Paris.

Phone: +331 40 51 64 34; Fax: +331 40 51 64 30; e-mail: ralf.jockers@inserm.fr

Virginie Vauthier^{1,2,3}, Sylvie Jaillard^{4,5}, Hubert Journal⁶, Christèle Dubourg^{4,7}, Ralf Jockers^{1,2,3,*}, Julie Dam^{1,2,3}

¹ Inserm, U1016, Institut Cochin, Paris, France

² CNRS UMR 8104, Paris, France

³ Univ. Paris Descartes, Sorbonne Paris Cité, Paris, France

⁴ Institut de Génétique et Développement, CNRS UMR6290, Université de Rennes 1, IFR140 GFAS, Faculté de Médecine, Rennes, France

⁵ Laboratoire de Cytogénétique, CHU Pontchaillou, Rennes Cedex, France.

⁶ Service de Génétique Médicale, Centre Hospitalier Bretagne Atlantique, Vannes, France

⁷ Laboratoire de Génétique Moléculaire, CHU Pontchaillou, Rennes Cedex, France

Running title: Homozygous deletion of the *DNAJ6C* and the *LEPR* gene

Key words: leptin receptor, OB-RGRP/endospanin-1, epilepsy, obesity, auxilin-1

Abstract:

Context: Homozygous mutations within *LEPR*, the leptin receptor (OB-R) gene, are rare and cause early-onset obesity. The genomic organization of the *LEPR* gene is complex and generates three independent transcripts whose respective functions are still poorly understood.

Methods/Results: We describe here a 7-year old patient with a homozygous 80 kb deletion in the chromosomal 1p31.3 region with early onset obesity, mental retardation and epilepsy. The deleted region comprises the proximal promoter and exons 1 and 2 of the *LEPR* gene and exons 5 to 19 of the *DNAJ6C* gene. The deletion leads to the deficiency of all canonical OB-R isoforms but maintains the B219 OB-R short isoforms controlled by the preserved second *LEPR* promoter. The *DNAJ6C* gene encodes auxilin-1, a protein required for clathrin-dependent recycling of synaptic vesicles in neurons that is possibly at the origin of the mental retardation and epilepsy phenotype. The obese phenotype and the absence of signaling-competent OB-R are consistent with previously reported individuals with OB-R deficiency. The deletion eliminates an additional transcript of the *LEPR* gene that encodes endospanin-1, a protein that has been genetically and biochemically linked to OB-R function. **Conclusions:** Our study confirms the phenotype of individuals with OB-R deficiency and postulates for the first time the effects of auxilin-1 deficiency (mental retardation/epilepsy) and endospanin-1 deficiency (OB-R specific functions) in humans.

Introduction

Obesity is one of the greatest current public health challenges, not only in industrialized countries but also in developing countries (1). The obesity epidemic is driven by recent lifestyle and environmental changes together with the genetic heritage of each individual. Leptin has been identified as a key hormone of body weight regulation and energy homeostasis that acts on leptin receptors (OB-R) that are primarily expressed in the hypothalamus but also in other central and peripheral regions (2). The central role of leptin and its receptor is illustrated by the obese phenotype of rodents and humans carrying non-functional mutations in the *ob* and *LEPR* genes encoding leptin and its receptor, respectively (3, 4). To date, only 10 families have been reported with mutations in the *LEPR* gene (5-7). Homozygous carriers of these mutations show early onset obesity, hyperphagia, altered immune function and hypogonadotropic hypergonadism with the lack of pubertal growth spurt.

The genomic organization of the *LEPR* gene is complex. The P1 promoter of the *LEPR* gene generates two distinct transcripts through alternative splicing, the OB-R transcript and the Leptin Receptor Overlapping Transcript (LEPROT) that share the same 5' untranslated region but use different AUG initiation codons giving rise to two distinct open reading frames (Fig. 1A) (8, 9). The OB-R transcript encodes different OB-R protein isoforms including the signaling-competent long OB-Rb isoform. The LEPROT transcript encodes OB-R gene-related protein (OB-RGRP) or also called endospinin-1, which has no sequence homology with OB-R (8). Furthermore, OB-R/B219, a third transcript, is expressed from a second, internal, P2 promoter (Fig. 1A). This transcript generates different short OB-R isoforms (B219), with an expression pattern that is different from the receptors originating from the OB-R transcript (giving rise to long and short isoforms). Despite earlier suggestions that OB-R isoforms generated by the OB-R/B219 transcript might be involved in hematopoiesis (10), their function remains still largely unknown.

Recently it has been shown that Endospinin-1 regulates the number of OB-R at the cell surface *in vitro* by retaining the receptor in intracellular compartments through an interaction with OB-R (9, 11). The

phenotype of knockout mice for endospinin-1 has not been described so far. Interestingly, shRNA-mediated silencing of endospinin-1 specifically in the hypothalamic arcuate nucleus improves OB-R-dependent signaling and prevents against the development of diet-induced obesity (11).

We describe here the phenotype of a 7-year-old boy carrying a homozygous deletion of a 80 kb region at chromosomal 1p31.3 region that includes most of the coding exons of *DNAJ6C* gene and the 5' part of the *LEPR* gene resulting in the abolishment of auxilin-1, OB-R and LEPROT transcripts but the maintenance of the OB-R/B219 transcript.

Case History And Clinical Examination

In 2004, a 3 year old male patient with early severe obesity was referred to the Genetic Counseling Unit, Vannes, France, to investigate his obesity-associated hyperphagia and developmental delay (fine motor problems associated with language learning difficulties). By 7 years old, he was 122cm tall and weighed 41 kg, with a BMI of 27.5 kg/m² thus above the 99th percentile defining a state of obesity (Fig. 1B-D). The patient was born with a birth weight of 4090g, which is at the upper limit of normal birth weight (Fig. 1B). Starting from the age of 1, the BMI of the patient was higher than normal, mainly due to increased body weight (Fig. 1C) associated with elevated food-seeking behavior.

Clinical examinations of the patient (III1) showed normal height evolution until the age of 11 years. He exhibited mild dysmorphic features (round face, mild brachydactyly, undescended testis), obesity, mental retardation and epilepsy, but with no other clinical abnormalities. Past family history revealed that most members were overweight or obese (Fig. 1E, Table 1) and the patient's maternal grandmother (I2) as well as two paternal aunts (II1, II2) showed an epileptic history. The two latter (II1, II2) exhibited also mild mental retardation. However, in the present study, we were unable to further explore individuals from the paternal family.

The patient showed normal levels of serum lipids and hormones (Table 2). The number of blood cells was normal with the exception of low CD4⁺ T lymphocytes and increased CD8⁺ T lymphocytes that results in

a low CD4/CD8 T-cell ratio. The number NK lymphocytes was higher than normal (Fig. 2A). A summary of the features of mental retardation and the epileptic syndrome of III1 are presented in Supplementary table 1.

Materials and Methods

Blood parameters

Plasma levels of hormones and lipids were determined by standard methods and immunophenotyping by flow cytometry with a FACS Calibur (BECTON –DICKINSON).

Array-CGH

Oligonucleotide array-CGH was performed using the Agilent Human Genome CGH microarray 44K (Agilent Technologies, Santa Clara, CA, USA). These microarrays comprise more than 44,000 60-mer oligonucleotide probes that span both coding and non-coding regions. Microarrays were scanned using the Agilent scanner G2565BA. Data were analyzed with Agilent DNA Analytics software to identify chromosome aberrations. Identification of probes with a significant gain or loss was based on the log₂ ratio plot deviation from 0 with cut-off values of 0.5 to 1 or -0.5 to -1 respectively. A value of -4 characterise a homozygous deletion.

Multiplex PCR/Liquid chromatography (MP-LC)

Duplex PCR was performed, associating unlabeled primers for an endogenous control gene, *HMBS*, and for *OB-R/LEPR* showing imbalance in the patient. Duplex for normal control, patient and parents gDNAs was performed as described elsewhere (12, 13). Data analysis was performed using the Navigator™ Software (Transgenomic, Omaha, NE, USA), normalisation was achieved with *HMBS* peak and relative peak intensities directly reflected *OB-R/LEPR* genomic copy number.

DNA extraction, RNA extraction and semi quantitative RT-PCR

DNA and RNA extractions and RT-PCR experiments were performed according to standard procedures (for primers see [supplementary table 2](#)).

Results

Blood chromosome analysis showed that the subject (III1) had a normal karyotype. Initial genetic tests excluded X Fragile Syndrome, the most common inherited cause of intellectual disability associated in some cases with obesity in males. Genetic tests on paternal chromosome 15q11-q13 as well as III1 clinical features (no hypotonia, no genital hypoplasia, no short stature) excluded Prader-Willi syndrome which is a complex genetic condition also characterized with obesity and intellectual impairment (data not shown). Array-CGH analysis revealed a chromosome 1 interstitial homozygous deletion on 1p31.3, confirmed by dye-swap (Fig. 1F) and excluded any other micro-deletion in the genome. The distal breakpoint mapped within the chromosome interval 65.604 Mb (the last non deleted probe) and 65.627 Mb (deletion starting point) from 1p telomere and the proximal one within the chromosome interval 65.707 (deletion ending point) and 65.744 Mb (first non deleted probe). Hence the deletion encompasses about 80 kilobases. To confirm array data, multiplex PCR/Liquid Chromatography (MPLC) experiments were performed for III1 and his parents. The homozygous deletion was confirmed in III1, inherited from asymptomatic and probably consanguineous parents, as each were heterozygous for the same deletion (Fig. 1G). The 80 kilobases deletion comprises only two known genes: (i) the *DNAJ6C* gene, encoding auxilin-1, a protein predominantly expressed in the brain that is required for clathrin recycling in cells (14), and (ii) the *LEPR* gene, known to encode the leptin receptor (OB-R) (15) and endospanin-1 (8, 9). The deletion encompasses the 3' region of the *DNAJ6C* gene starting from *DNAJ6C* exon 5 and includes the 5' part of the *LEPR* gene comprising the proximal P1 promoter region and exons 1 and 2 (Fig. 1A,

1F). MPLC analysis showed that both parents are heterozygous for the 1p31.3 deletion (Fig. 1G) and that 6 other related family members exhibited the same 1p31.3 deletion (Fig. 1E, 1G).

To further confirm the absence of the 1p31.3 region in III1, specific PCR primers were designed to amplify this region in III1 and other family members (Fig. 1A, Supplementary Fig. 1A). As expected, primers specific for *DNAJ6C* gene exon 9 and for *LEPR* gene exon 2 readily revealed the expected amplicon in heterozygous family members but not in III1.

The deletion of 91% of *DNAJ6C* coding region is in accordance with the absence of functional auxilin-1 transcript in III1 as compared to wild type or heterozygous carriers (Fig. 2B).

The presence of *LEPR* gene exon 8 was detected in all cases consistent with the partial deletion of the *LEPR* gene. The corresponding mRNAs for auxilin-1, LEPROT and OB-R were absent in blood samples of III1 but present in heterozygous family members (Fig. 2B). Similar results were obtained in skin mRNA preparations (Supplementary Fig. 1B).

The presence of OB-R/B219 transcript generated from the internal P2 promoter was confirmed in mRNA samples prepared from III1 blood cells (Fig. 2B). Expression in blood cells but not in skin cells (data not shown) is in accordance with previous reports (8, 10).

Endospanin-1 has a unique homologue in the human genome called endospanin-2 that is encoded by the *LEPROTL1* gene located on chromosome 8p21.1-8p21.2 (9, 16). As recent studies in transfected cells suggest that both endospanins negatively regulate OB-R surface expression, a compensatory effect of one endospanin in the absence of the other might be possible. However, semi-quantitative RT-PCR analysis showed that similar amounts of endospanin-2 mRNA were present in blood samples of III1, heterozygous carriers and wild-type family members (Supplementary Fig. 1C, Fig. 2C).

Discussion

In the present study, we report for the first time a patient (III1) with a homozygous deletion within the chromosomal 1p31.3 region comprising only two predicted genes, *DNAJ6C* and *LEPR* genes. Whereas III1 with the homozygous deletion showed early on-set obesity, mental retardation and epilepsy, 8 heterozygous carriers including a sibling of III1 are also overweight or obese, but do not exhibit mental retardation or epilepsy (except 1 member).

DNAJ6C encodes auxilin-1, a protein expressed in the brain and acting as a co-chaperone to support Hsc70-dependent clathrin uncoating of clathrin-coated vesicles in neurons (14). Mice with targeted deletion of auxilin-1 show recycling and endocytosis defects of synaptic vesicles and are characterized by early postnatal mortality likely due to neurological and cognitive impairment (17). Recently, major reports demonstrated that, in mice and humans, deficiency of genes involved in synaptic vesicle recycling and endocytosis lead to impairment of neurocognitive functions characterized by mental retardation, defect in learning/memory and seizures: endophilin (18), oligophrenin-1 (19-21), dynamin-1 (22), amphiphysin-1 (23), synaptojanin-1 (24, 25). Importantly, given the widespread impact of synaptic neurotransmission on brain functions, many of these deficiencies led to perinatal mortality due to severe neurologic defects (17, 18, 23-25). We propose that auxilin-1 could be a new potential candidate gene for mental retardation and/or seizure, which requires further investigation in other human genetic studies.

Deletion of exons 1-2 and of P1 promoter of *LEPR* gene eliminates OB-R and LEPROT transcripts but not the OB-R/B219 transcript, which uses the internal P2 promoter. The phenotype of previously reported patients with non-functional OB-R resembles that of III1 described in our study: early onset obesity, hyperphagia and modification of the immune cells. Moreover, the high circulating leptin level of 50 ng/mL in the patient (normal range for boys of similar age is below 11.2 ng/mL), may explained the absence of a putative negative feedback loop between leptin signaling and leptin secretion. Consistently, decreased CD4/CD8 T lymphocyte ratios have also been observed in humans with congenital leptin

deficiency (26). The lack of pubertal growth spurt and hypogonadotropic hypergonadism reported for patients with leptin or OB-R deficiency has not yet been observed, as III1 is too young. Thus, obesity and defects in immune cells observed in III1 are most likely explained by the absence of the signaling-competent long OB-Rb isoform. Mental retardation and epilepsy are unlikely to be causally related to the absence of OB-Rb as other reported mutations of the leptin pathway do not present this phenotype.

Whereas previously described mutations of the *LEPR* gene concern all OB-R and OB-R/B219 transcripts equally, deletion of *LEPR* gene exons 1 and 2, identified in our study, only abolishes OB-Ra/b transcript leaving the OB-R/B219 transcripts fully functional. Interestingly, the obese phenotype and the modified CD4+/CD8+ T-cell ratio observed in III1 supports the idea that OB-R/B219 transcripts are not likely to be involved in these functions.

Some complex pleiotropic syndromes in which obesity is only one of the clinical phenotype of developmental anomalies are largely described (27). Many of the clinical phenotypes characterizing these syndromes were not seen in III1 and his family. Moreover, genetic analysis did not confirm the presence of X Fragile syndrome and Prader-Willi syndrome in III1. Human deficiency of BDNF (28) or of its cognate receptor TrkB (29) was also shown to trigger severe obesity, intellectual abilities and seizures. However, III1 does not likely have such mutations since BDNF or TrkB subjects also developed hyperactivity, impairment of nociception, hypotonia which were not observed in III1.

This is the first study reporting the homozygous deletion of the LEPROT transcript of the *LEPR* gene. No other mutations have been reported so far for this transcript in humans. Endospinin-1, the corresponding protein of the LEPROT transcript, has been recently shown to regulate OB-R function (9, 11). However, due to the simultaneous absence of the signaling-competent OB-Rb isoform in III1, we are unable to confirm this prediction. Normal IGF1 levels and normal growth of III1 also excludes any OB-R-independent effects on these parameters as suspected from a transgenic mouse model over-expressing

endospanin-1 (30). Our study allows us to postulate a preliminary phenotype of endospanin-1 deficiency in humans. As the phenotype of III1 is likely to be dominated by the absence of auxilin-1 (mental retardation, epilepsy) and OB-Rb (early on-set obesity, modified immune cell composition), it is likely that the absence of endospanin-1 has no major phenotypic consequences in humans in the context of OB-Rb deficiency. Co-evolution of both transcripts under the control of the same promoter further supports this conclusion.

In conclusion, we describe here a unique homozygous 80 kb deletion in the chromosomal 1p31.3 region that confirms the early onset obesity of individuals with OB-R deficiency and suggests for the first time the phenotype of auxilin-1 and endospanin-1 deficiency in humans.

Acknowledgements: This work was supported by grants from the FP7 “EUROCHIP”, Institut National de la Santé et de la Recherche Médicale (INSERM), Centre National de la Recherche Scientifique (CNRS), Direction de l'Hospitalisation et de l'Organisation des Soins (DHOS) and a research fellowship of the Fondation pour la Recherche Médicale (to VV). We thank Drs Y. Cano, A. Bouvet, P Pouedras and teams from the “Service de Pédiatrie” (Centre Hospitalier Bretagne Atlantique,), Dr V. Gauthier (CHU, Nantes), Pr J. Mosser (Biogenouest transcriptomic platform, Rennes), K. Kerouredan (Centre d'action médico-sociale précoce (CAMSP), Vannes) for help with the family studies. We gratefully thank the subject and his family for their consent and participation.

Contributions: V.V. and S.J. performed research and analyzed data; R.J. and J.D. wrote the manuscript; V.V., C.D. and H.J. reviewed/edited the manuscript and contributed to discussion; R.J., H.J., J.D. and C.D. managed the project, H.J. performed medical examinations.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

References

1. **Bonow RO, Eckel RH** 2003 Diet, obesity, and cardiovascular risk. *N Engl J Med* 348:2057-2058
2. **Myers MG, Jr., Leibel RL, Seeley RJ, Schwartz MW** 2010 Obesity and leptin resistance: distinguishing cause from effect. *Trends Endocrinol Metab* 21:643-651
3. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM** 1994 Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425-432
4. **Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, et al** 1995 Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83:1263-1271
5. **Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gormelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lebouc Y, Froguel P, Guy-Grand B** 1998 A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 392:398-401
6. **Farooqi IS, Wangensteen T, Collins S, Kimber W, Matarese G, Keogh JM, Lank E, Bottomley B, Lopez-Fernandez J, Ferraz-Amaro I, Dattani MT, Ercan O, Myhre AG, Retterstol L, Stanhope R, Edge JA, McKenzie S, Lessan N, Ghodsi M, De Rosa V, Perna F, Fontana S, Barroso I, Undlien DE, O'Rahilly S** 2007 Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *N Engl J Med* 356:237-247
7. **Mazen I, El-Gammal M, Abdel-Hamid M, Farooqi IS, Amr K** 2011 Homozygosity for a novel missense mutation in the leptin receptor gene (P316T) in two Egyptian cousins with severe early onset obesity. *Mol Genet Metab* 102:461-464
8. **Bailleul B, Akerblom I, Strosberg AD** 1997 The leptin receptor promoter controls expression of a second distinct protein. *Nucleic Acids Res* 25:2752-2758
9. **Seron K, Couturier C, Belouzard S, Bacart J, Monté D, Corset L, Bocquet O, Dam J, Vauthier V, Lecœur C, Bailleul B, Hoflack B, Froguel P, Jockers R, Rouillé Y** 2011 Endospanins regulate a post-internalization step of leptin receptor endocytotic pathway. *J Biol Chem* 286:17968-17981
10. **Cioffi JA, Shafer AW, Zupancic TJ, Smith-Gbur J, Mikhail A, Platika D, Snodgrass HR** 1996 Novel B219/OB receptor isoforms: possible role of leptin in hematopoiesis and reproduction. *Nat Med* 2:585-589
11. **Couturier C, Sarkis C, Seron K, Belouzard S, Chen P, Lenain A, Corset L, Dam J, Vauthier V, Dubart A, Mallet J, Froguel P, Rouille Y, Jockers R** 2007 Silencing of OB-RGRP in mouse hypothalamic arcuate nucleus increases leptin receptor signaling and prevents diet-induced obesity. *Proc Natl Acad Sci U S A* 104:19476-19481
12. **Bendavid C, Dubourg C, Pasquier L, Gicquel I, Le Gallou S, Mottier S, Durou MR, Henry C, Odent S, David V** 2007 MLPA screening reveals novel subtelomeric rearrangements in holoprosencephaly. *Hum Mutat* 28:1189-1197
13. **Jaillard S, Dubourg C, Gerard-Blanluet M, Delahaye A, Pasquier L, Dupont C, Henry C, Tabet AC, Lucas J, Aboura A, David V, Benzacken B, Odent S, Phipras E** 2009 2q23.1 microdeletion identified by array comparative genomic hybridisation: an emerging phenotype with Angelman-like features? *J Med Genet* 46:847-855
14. **Lemmon SK** 2001 Clathrin uncoating: Auxilin comes to life. *Curr Biol* 11:R49-52
15. **Tartaglia LA** 1997 The leptin receptor. *J Biol Chem* 272:6093-6096
16. **Huang Y, Ying K, Xie Y, Zhou Z, Wang W, Tang R, Zhao W, Zhao S, Wu H, Gu S, Mao Y** 2002 Cloning and characterization of a novel human leptin receptor overlapping transcript-like 1 gene (LEPROTL1). *Biochim Biophys Acta* 1517:327-331

17. **Yim YI, Sun T, Wu LG, Raimondi A, De Camilli P, Eisenberg E, Greene LE** 2010 Endocytosis and clathrin-uncoating defects at synapses of auxilin knockout mice. *Proc Natl Acad Sci U S A* 107:4412-4417
18. **Milosevic I, Giovedi S, Lou X, Raimondi A, Collesi C, Shen H, Paradise S, O'Toole E, Ferguson S, Cremona O, Camilli PD** 2011 Recruitment of endophilin to clathrin-coated pit necks is required for efficient vesicle uncoating after fission. *Neuron* 72:587-601.
19. **des Portes V, Boddaert N, Sacco S, Briault S, Maincent K, Bahi N, Gomot M, Ronce N, Bursztyjn J, Adamsbaum C, Zilbovicius M, Chelly J, Moraine C** 2004 Specific clinical and brain MRI features in mentally retarded patients with mutations in the Oligophrenin-1 gene. *Am J Med Genet A* 124A:364-71.
20. **Nakano-Kobayashi A, Kasri NN, Newey SE, Van Aelst L** 2009 The Rho-linked mental retardation protein OPHN1 controls synaptic vesicle endocytosis via endophilin A1. *Curr Biol* 19:1133-9.
21. **Pirozzi F, Di Raimo FR, Zanni G, Bertini E, Billuart P, Tartaglione T, Tabolacci E, Brancaccio A, Neri G, Chiurazzi P** 2011 Insertion of 16 amino acids in the BAR domain of the oligophrenin 1 protein causes mental retardation and cerebellar hypoplasia in an Italian family. *Hum Mutat* 32:E2294-307.
22. **Boumil RM, Letts VA, Roberts MC, Lenz C, Mahaffey CL, Zhang ZW, Moser T, Frankel WN** 2010 A missense mutation in a highly conserved alternate exon of dynamin-1 causes epilepsy in fitful mice. *PLoS Genet.* 6(8).
23. **Di Paolo G, Sankaranarayanan S, Wenk MR, Daniell L, Perucco E, Caldarone BJ, Flavell R, Picciotto MR, Ryan TA, Cremona O, De Camilli P** 2002 Decreased synaptic vesicle recycling efficiency and cognitive deficits in amphiphysin 1 knockout mice. *Neuron* 33:789-804.
24. **Cremona O, Di Paolo G, Wenk MR, Lüthi A, Kim WT, Takei K, Daniell L, Nemoto Y, Shears SB, Flavell RA, McCormick DA, De Camilli P** 1999 Essential role of phosphoinositide metabolism in synaptic vesicle recycling. *Cell* 99:179-88.
25. **Kim WT, Chang S, Daniell L, Cremona O, Di Paolo G, De Camilli P** 2002 Delayed reentry of recycling vesicles into the fusion-competent synaptic vesicle pool in synaptojanin 1 knockout mice. *Proc Natl Acad Sci U S A.* 99:17143-8.
26. **Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S, Lechler RI, DePaoli AM, O'Rahilly S** 2002 Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest* 110:1093-1103
27. **O'Rahilly S, Farooqi IS, Yeo GS, Challis BG** 2003 Minireview: human obesity-lessons from monogenic disorders. *Endocrinology* 144:3757-64.
28. **Gray J, Yeo GS, Cox JJ, Morton J, Adlam AL, Keogh JM, Yanovski JA, El Gharbawy A, Han JC, Tung YC, Hodges JR, Raymond FL, O'rahilly S, Farooqi IS** 2006 Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. *Diabetes* 55:3366-71.
29. **Yeo GS, Connie Hung CC, Rochford J, Keogh J, Gray J, Sivaramakrishnan S, O'Rahilly S, Farooqi IS** 2004 A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. *Nat Neurosci* 7:1187-9.
30. **Touvier T, Conte-Auriol F, Briand O, Cudejko C, Paumelle R, Caron S, Bauge E, Rouille Y, Salles JP, Staels B, Bailleul B** 2009 LEPROT and LEPROTL1 cooperatively decrease hepatic growth hormone action in mice. *J Clin Invest* 119:3830-3838

Figure legends

Figure 1. Deletion of chromosomal 1p31.3 region comprising the *DNAJ6C* and *LEPR* gene. **A.** Organization of *DNAJ6C* and *LEPR* genes and corresponding transcripts. *LEPR* gene expression is controlled by a dual promoter: the P1 promoter generates two distinct transcripts (Leptin Receptor Overlapping Transcript (LEPROT) and OB-R) through alternative splicing giving rise to two distinct proteins without any amino acid sequence homology (OB-RGRP/endospanin-1 or OB-R short and long isoforms, respectively) and the alternative P2 promoter allows the expression of B219 OBR short isoforms. Positions of primers used for genomic DNA (dark grey arrows) and mRNA (soft grey arrows) amplification and AUG allowing protein translation are indicated. **B-D.** Patient's height (**B**), body weight (**C**) and Body Mass Index (BMI) (**D**) from age 1 to age 10. According to the definition of childhood obesity in France, 97th and 99th percentile BMI cutoff points characterize moderate and severe obesity respectively (equivalent to threshold defining childhood "overweight" or "obesity" according to the International Obesity task Force (IOTF) respectively). **E. Pedigree chart of the consanguineous family.** The squares represent male family members and the circles female family members; open symbols represent wild type family members and solid symbols are members with deleted allele (grey for heterozygous; black for homozygous). **F. The patient's Array-CGH data.** The left part of the figure shows chromosome 1 ideogram. Each dot represents a single probe spotted on the array. Dots with a value of zero represent equal fluorescence intensity ratio between patient sample and reference genomic DNAs. Copy number losses shift the ratio to the left or to the right according to the fluorescent labelling. The deletion revealed by array-CGH is highlighted (black rectangle) and dye reversal hybridization experiment shows a mirror image. The right part of the figure, with the log₂ ratio of chromosome 1 probes, zooms in on this micro-rearrangement and shows which genes are contained in the deletion. **G. Multiplex PCR/Liquid Chromatography validation experiment.** The electrophoregrams of the patient (in red) and of the parents (mother in green, father in blue) were superimposed to those of controls (in

black) and normalized on *HMBS* internal control peak (132 bp) intensity. The presence of a heterozygous or homozygous deletion was indicated by a twofold reduction in the height of *OB-R/LEPR* amplicon peak (118 bp) or by a flat profile respectively.

Figure 2: Characterization of gene expression in 5 family members. **A.** T, B and NK cell population counts (absolute values in giga cells/L). **B.** Semi-quantitative RT-PCR from mRNA extracted from blood cells. **C.** Quantification of endospainin2 mRNA level from Supplem Fig. 1C with ImageJ. GAPDH and HPRT genes are used as internal control. * indicates out of normal range.

Table 1: General phenotype of the family members including age, genotype, height, body weight (BW), BMI and other diseases. values[§] to be compared to normal range of childhood BMI which are age-dependant in children (see Fig. 1D).

Table 2: Metabolic and endocrine features of 4 family members including the patient (III1) and its 2 sisters. Age and sex-specific normal ranges for leptin 2.4-11.2 ng/mL for 3-11 years old, <14.8 for women, <7.4 for men; for estradiol, < 0.076 pmol/L for clinically prepubertal persons, 0.01-0.75 for women in follicular phase; for testosterone, <1.43 ng/mL for clinically prepubertal persons; for Insulin-like Growth factor 1 (IGF-1), 6-39 for < 10 years old, 15-130 for 10-13 years old, 10-51 for > 13 years old; for Follicle-Stimulating hormone (FSH), <1U/L for clinically prepubertal subjects, 1-18 for pubertal subjects; for Luteinizing Hormone (LH) < 1U/L for clinically prepubertal subjects, 1.5-13 for pubertal subjects. * indicates out of normal range.

Supplementary figure 1: A. PCR on genomic DNA extracted from blood cells. **B.** Semi-quantitative RT-PCR from mRNA extracted from skin biopsies. **C.** Semi-quantitative RT-PCR from mRNA extracted from blood cells

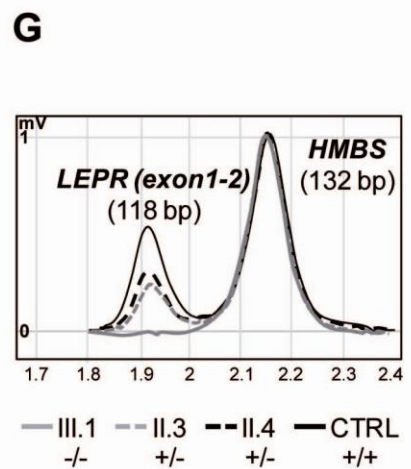
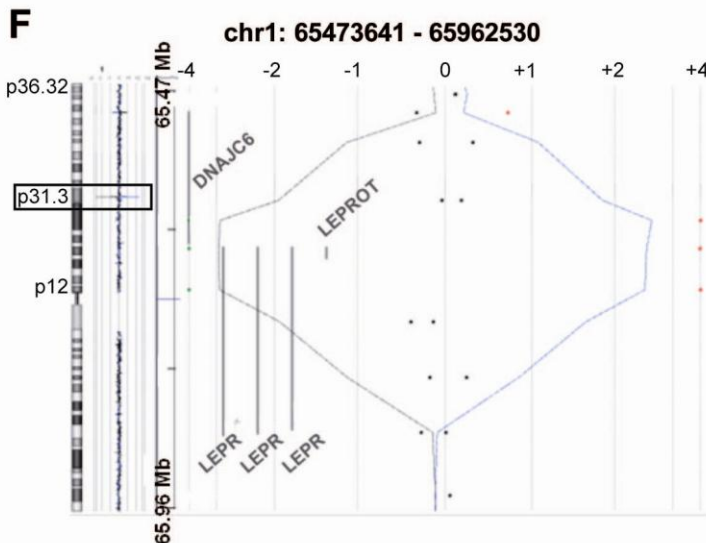
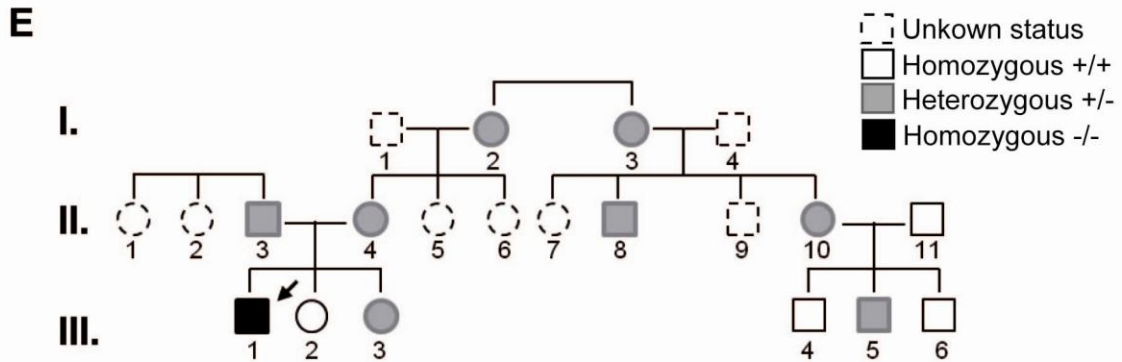
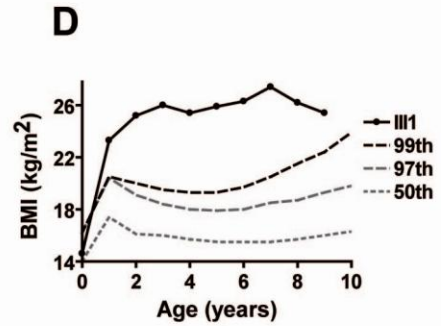
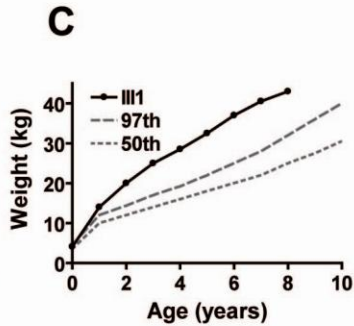
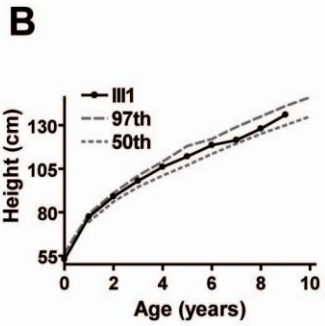
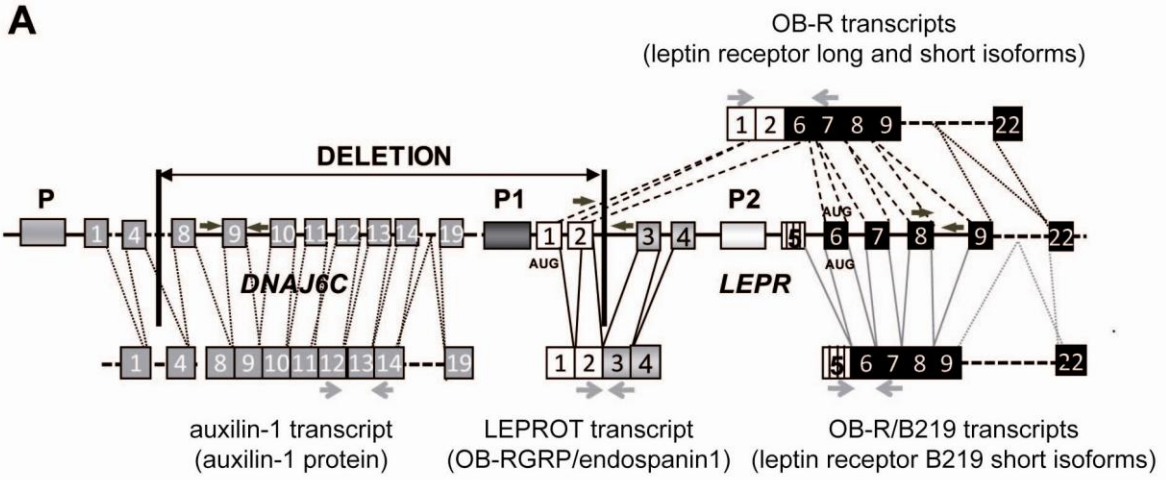


Figure 1

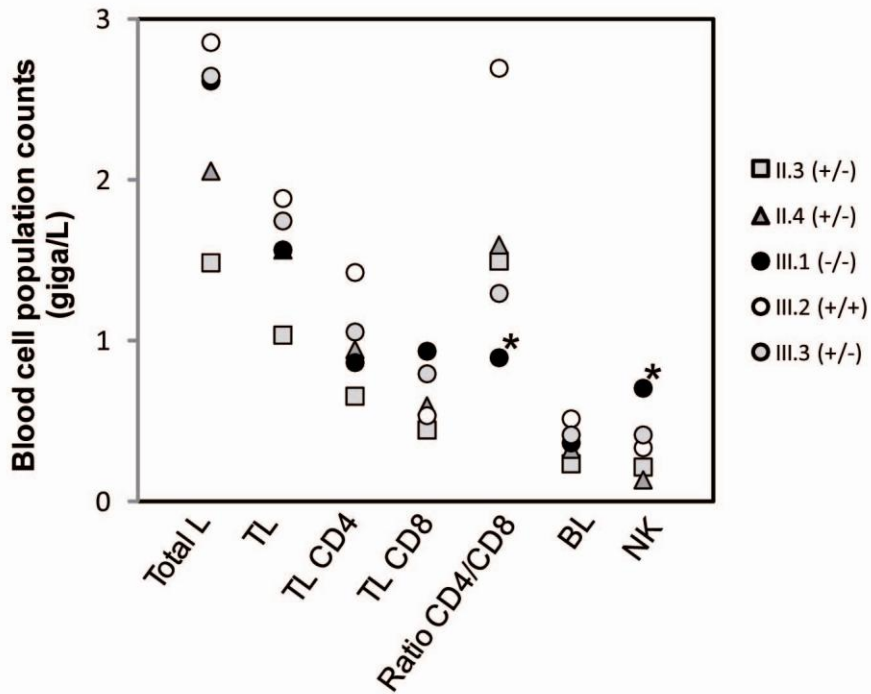
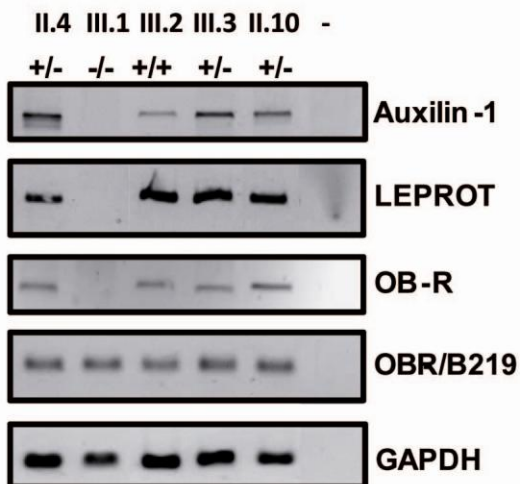
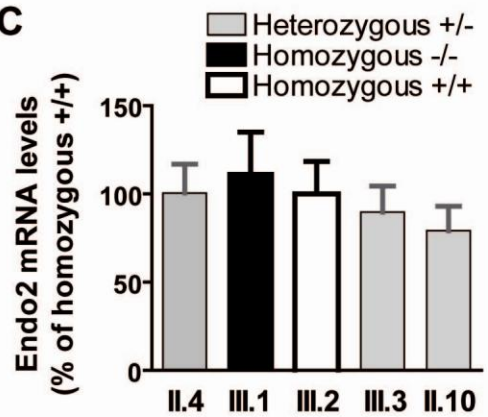
A**B****C**

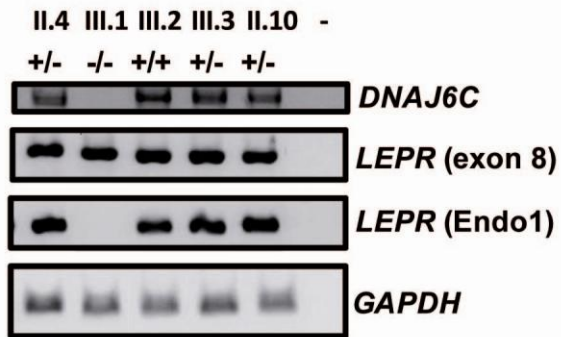
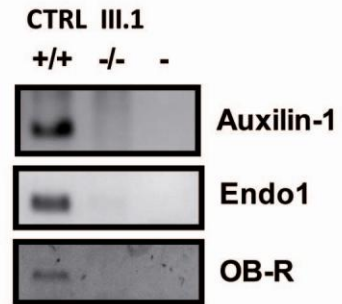
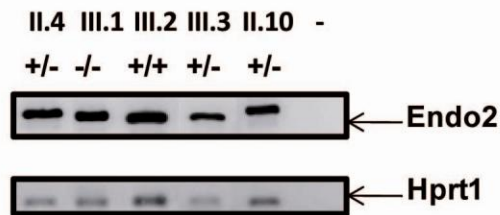
Figure 2

Table 1: General phenotype of the family members including age, genotype, size, body weight (BW), BMI and other diseases.

Ind.	Age	Genotype	Size (cm)	BW (kg)	BMI (kg/m ²)	BW Phenotype	Particularity
I.2	52	+/-	155	75	31.2	obese	Epilepsy
I.3	56	+/-	158	70	28	overweight	
II.1	-	-	-	-	-	-	Epilepsy/mild mental retardation
II.2	-	-	-	-	-	-	Epilepsy/mild mental retardation
II.3	36	+/-	168	68	24.1	normal	
II.4	32	+/-	165	70	25.5	overweight	
II.8	33	+/-	170	95	32.9	obese	
II.10	29	+/-	165	115	43.8	obese	
II.11	36	+/+	166	75	27.2	overweight	
III.1	7	-/-	122	41	27.5[§]	obese	Epilepsy Mental retardation
III.2	4	+/+	107	20	17.8 [§]	normal	
III.3	12	+/-	151	50	21.9 [§]	overweight	Deafness
III.5	5	+/-	110	25	20.7 [§]	obese	

Table 2: Metabolic and endocrine features of 5 family members including the patient (III1), his parents (II3 and II4) and his 2 siblings (III2 and III3).

	II.3	II.4	III.1	III.2	III.3	Normal Range
Genotype	+/-	+/-	-/-	+/+	+/-	
Weight at birth (g)	3800	3560	4090	3760	3440	
Height at birth (cm)	-	50.5	53	51	50	
Age (years)	36	32	7	4	12	
BMI (kg/m ²)	24.1	25.5	27.5*	17,8	21,9*	
Leptin (ng/mL)	3.1	18	50*	5.7	33*	Age-specific
Glycemia (mmol/L)	5.1	4.9	5.1	4.8	5.2	3.9 – 5.8
Blood platelet (giga/L)	169	223	281	354	261	150-400
Estradiol (nmol/L)	0.09	72	0.05	0.02	0.13	< 0,07
Insulin (pmol/L)	34	41	106	30	74	13 - 161
Somatotrophin HgH (mUI/L)	<0.2	3	1.8	2.3	1.3	M <2.9 F: < 10.8
IGF1 (nmol/L))	15	24	22	19	52	Age-specific
Testosterone (ng/mL)	-	-	0.2	0.2	0.7	Age-specific
FSH (U/L)	3.2	14.1	<0.2	0.5	5.1	Age-specific
LH (U/L)	5	7.8	<0.2	<0.2	2.4	Age-specific
Free thyroxine T4(pmol/L)	16.4	15.3	15.8	20.5	14.7	11.5 - 22.7
TSH (mU/L)	2.35	1.75	3.91	3.29	2.85	0.48 - 4.7
Cholesterol (mmol/L)	4.08	4.79	4.19	4.88	4.84	< 5.2
Triglycerides (mmol/L)	1.78	0.58	0.69	0.67	0.91	0.3 – 1.6

A**B****C**

Supplementary Table 1: Clinical survey: Motor and speech disabilities and epilepsy

Age	Development	Epilepsy
month 3	Head held up	
month 6		Hyperthermic seizures
month 9	Sits unsupported	
month 20	Walks with support	Hyperthermic seizures
year 2.5	Walks alone but unstable, No language	1 Grand Mal Seizure
year 3.5	Climbing stairs by crawling, unable to jump	1 Grand Mal Seizure
year 5	Poor language, unstable walk	Epileptic encephalopathy with diffuse slow spike-waves
year 6		3 short seizures (<15 minutes)
year 8		Tonic-clonic seizures (45 minutes)
year 9		Tonic-clonic seizures (30 minutes)
year 10	IQ = 40	2 Tonic-clonic seizures (>2hours) and (3 hours)

Supplementary Table 2: Primer sequences

Gene	Primers Sequences
Genomic DNA	
DNAJ6C	5'-GTTTACCCTTATTCCCTCAC-3' 5'-AGTTTGTTTTAGCCAGCAGT-3'
<i>LEPR</i>	5'-GGACAACCAGCCAAAGGAC-3' 5'-CAGGTGAGGGGCAAGAGACT-3'
GAPDH	5'-GATGGCATGGACTGTGG-3' 5'-CCCGCTCCCTCTTTCTTTG-3'
Transcripts	
Auxilin-1	5'-TCAGAAATCGGAGAAGTCAT-3' 5'-AGAGGTGGAGGTGGCAGAGC-3'
OB-R	5'-AGCAGCCGCGGCCCCAGTTC-3' 5'-TGACAAGTTAAACGCAGTTATCACAT-3'
LEPROT	5'-GAGGAGGGGCTACCACTGTTC-3' 5'-GGGCTGACTTTTCTTATGCT-3'
OB-R/B219	5'-TGACAAGTTAAACGCAGTTATCACAT-3' 5'-AGCAGCCGCGGCCCCAGTTC-3'
HPRT	5'-AGGACCTCTCGAAGTGT-3' 5'-ATTCAAATCCCTGAAGTACTCAT-3'
GAPDH	5'-GATGGCATGGACTGTGG-3' 5'-GGAGAAGGCTGGGGC-3'