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Functional and Phenotypic Characteristics of Human Leptin Receptor Mutations

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Several case series of extreme early-onset obesity due to mutations in the human leptin receptor (LEPR) gene have been reported. In this review we summarize published functional and phenotypic data on mutations in the human LEPR gene causing severe early-onset obesity. Additionally, we included data on six new cases from our obesity center. Literature research was performed using PubMed and OMIM. Functional relevance of mutations was estimated based on reported functional analysis, mutation size, and location, as well as phenotypic characteristics of affected patients. We identified 57 cases with 38 distinct LEPR mutations. We found severe early-onset obesity, hyperphagia, and hypogonadotropic hypogonadism as cardinal features of a complete loss of LEPR function. Other features, for example, metabolic disorders and recurring infections, were variable in manifestation. Obesity degree or other manifestations did not aggregate by genotype. Few patients underwent bariatric surgery with variable success. Most mutations occurred in the fibronectin III and cytokine receptor homology II domains, whereas none was found in cytoplasmic domain. *In silico* data were available for 25 mutations and *in vitro* data were available for four mutations, revealing residual activity in one case. By assessing provided information on the clinical phenotype, functional analysis, and character of the 38 mutations, we assume residual LEPR activity for five additional mutations. Functional *in vitro* analysis is necessary to confirm this assumption.

Freeform/Key Words: early-onset obesity, leptin melanocortin signaling pathway, leptin receptor deficiency, monogenic obesity

Leptin (LEP) and its receptor (LEPR) are key players in the regulation of body weight and energy homeostasis [1]. LEP is produced mainly in adipocytes, and blood levels are strongly correlated with the amount of body fat [2]. LEP regulates food intake and energy homeostasis by binding to its receptor on neurons in the hypothalamus [3]. Activation of LEPR on proopiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript neurons results in production of α-melanocyte–stimulating hormone that activates the melanocortin-4 receptor (MC4R) and thereby induces satiety signals. LEPR activation on neuropeptide-Y/agouti-related protein (AgRP) neurons leads to reduced production of orexigenic peptides [4, 5]. In 1998, Clément *et al.* [6] were the first to describe three subjects with severe early-onset

Abbreviations: aa, amino acid; AgRP, agouti-related protein; BMI, body mass index; CRH, cytokine receptor homology; *db*, diabetes mouse model; FNIII, fibronectin type III; JAK, Janus kinase; LEP, leptin; LEPR, LEP receptor; MC4R, melanocortin-4 receptor; NTD, N-terminal domain; *ob*, obese mouse model; POMC, proopiomelanocortin; sLEPR, soluble LEPR; STAT, signal transducer and activator of transcription.

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obesity due to a biallelic mutation in the *LEPR* gene (Clem_1.1 to 1.3). Since then, data on 48 other individuals with biallelic *LEPR* mutations have been published. The aim of this work is to summarize current knowledge on function and structure of the LEPR and to provide an overview of clinical and laboratory findings in patients with LEPR deficiency. To this end, we reviewed the literature and report six new cases followed at our obesity center.

1. Methods and Patients

We performed a literature search using PubMed and OMIM to find published cases with biallelic LEPR mutations (applied search terms in PubMed included LEPR, human LEPR, leptin-receptor, human leptin-receptor, monogenetic obesity, LEPR and obesity; 1 July 2017). We extracted clinical, biochemical, and functional data. Additionally, we included data obtained from n = 6 subjects with *LEPR* mutations who presented to our obesity center with severe early-onset obesity.

Ulm_1 is a boy from consanguineous parents from Turkey. He has a homozygous deletion of exons 4 to 20. The 7-year-old boy displayed a BMI of 33.2 kg/m^2 (*z* score +5.4) upon presentation.

Ulm_2 is the son of consanguineous parents of Turkish origin. He has two homozygous mutations in p.P316W and p.W646C. The boy presented with a BMI of 39.3 kg/m^2 (*z* score +6.1) at age 7 years.

Ulm_3 is a German boy born to parents without known consanguinity. He has the homozygous mutation p.H684P. At the age of 9.5 years, he had a BMI of 41.2 kg/m^2 (*z* score +4.5).

Ulm_4 is a German girl born to nonconsanguineous parents. She is compound heterozygous for p.S743P and c.2598-3_2607 deletion. At the age of 15.2 years, she had a BMI of 50.3 kg/m^2 (*z* score +4.0).

Ulm_5 is a 6-year-old girl born to consanguineous Turkish parents. She has the homozygous mutation p.N154Kfs*3. At the age of 5 years, her BMI was 46.1 kg/m^2 (*z* score +7.3)

Ulm_6 is compound heterozygous for p.W625* and p.H684P. At age 14 years, the boy of German origin had a BMI of 53.7 kg/m^2 (*z* score +4.1).

Ulm_1 to 6 or their legal guardian gave written consent to publish their data.

BMI *z* score calculations were made for subjects were no *z* score was published but values for age, weight, and height were available. Calculations are based on the World Health Organization standard and are provided for Ulm1-6, Clem_1.1 to 1.3, Maz_1-2, Vau_1, Bey_1, and Gil_1 [7, 8].

Figures were created using GraphPad Prism 6.07 (GraphPad Software, La Jolla, CA) and Inkscape (http://inkscape.org).

We converted all units to the SI system when possible. Using Ensembl.org we standardized and completed genomic information when necessary information was available. Mutation nomenclature is based on the recommendations made by the Human Genome Variation Society when all necessary information was available.

Estimations about functional impact of the respective mutations are based on the literature on LEPR function and by critical investigations of the phenotype, family background, and provided information about functional analysis. We defined five items for functionally relevant criteria: (1) highly suspicious BMI (BMI at 2 years of age >25, 5 years of age >30, and 10 years of age >40); (2) hypogonadotropic hypogonadism; (3) consanguineous parents; (4) highly suspicious variant (large deletion, frameshift, or mutation close to a functionally relevant region; mutation described in other subjects with similar phenotype); and (5) conclusive functional analysis (*e.g.*, *in vitro* analysis or Sanger sequencing and PCR to detect deletions. *In silico* analysis were not considered as conclusive analysis. Conclusions on functional relevance are based on the number of fulfilled criteria: “high” indicates high evidence for complete loss of LEPR function (three to five criteria fulfilled); “probably” indicates that the mutation is probably damaging (two to three criteria); “low” indicates low evidence for functional relevance, with in vitro analyses necessary to exclude residual function of LEPR (no to two criteria).

Supplemental Table 4 provides more information about the hypothesized molecular product resulting from the respective mutation: (1) truncated protein, not able to bind LEP; (2) sLEPR-like protein, able to bind LEP in the bloodstream; (3) membrane-anchored LEPR with no ability to bind LEP; and (4) membrane anchored LEPR with no ability for signal transduction.

We based our statements for genetic and protein data on the canonical transcript of the LEPR ENST00000349533.10/Transcript Variant NM_002303.5 using the database UniProt for information about the protein (http://www.uniprot.org/uniprot/P48357) and NCBI for the reference sequence (https://www.ncbi.nlm.nih.gov/nuccore/NM_002303.5).

2. Structure and Function of the LEPR

A. LEPR Isoforms

To date, four membrane-bound and one soluble isoform of the human LEPR have been described [9]. Notably, inconsistent nomenclature is used in the literature between and also within species (Table 1). LEPRb is encoded by the 20-exon canonical transcript. Its translation starts at exon 3 of the transcript. LEPRb is the only isoform with clearly defined functionality and is therefore the focus of most investigations [3]. Further studies are required to better understand the function of the other isoforms.

Structurally, all isoforms share the same extracellular domain with an 820–amino acid (aa) length (Fig. 1). The membrane-bound isoforms have a 21-aa transmembrane domain and a box 1 motif around positions 871 to 879, followed by variable C-terminal lengths due to alternative splicing from position 891 (Table 1). The soluble LEPR is a product of proteolytic ectodomain shedding [10]. It is the only described isoform without intracellular residues and a transmembrane domain and circulates in the bloodstream [11].

B. Receptor Structure and Function

The LEPR is a class I cytokine [Janus kinase (JAK)/signal transducer and activator of transcription (STAT)] receptor and shares many similarities with, for example, granulocyte colony-stimulating factor–, erythropoietin-, and oncostatin M–specific receptors [9, 12].

Table 1. LEPR Isoforms in Humans and Mice

Isoforms	Human	Mouse
Isoform A Alternative splicing	HuB or OB-R219.3: p.897–1165 missing p.892–896: PETFE → RTDIL	LEPRa: p.895–1162 missing p.890–894: PETFE → RTDTL
Isoform B Canonical sequence	HuB or OB-Rb/LEPRb: —	LEPRb: —
Isoform C Alternative splicing	HuB or OB-R219.1/LEPRa: p.907–1165 missing p.892–958: PETFEHLFIK ... EKGSVCISDQ → MLEGSMFVKS ... KSPSVRNTQE	LEPRc: p.893–1162 missing p.890–892: PET → VTV
Isoform D Alternative splicing	HuB or OB-R219.2: p.959–1165 missing p.892–906: PETFEHLFIKHTASV → KMPGTKELLGGGWLT	LEPRd: p.901–1162 missing p.890–900: PETFEHLFTKH → DISFHEVFIFR
Isoform E Alternative splicing	sLEPR: p.842–1165 missing	LEPRe/sLEPR: p.806–1162 missing p.797-805: DNFIPIEKY → GMCTVLFMD

Source: UniProt (mouse, http://www.uniprot.org/uniprot/P48356; human, http://www.uniprot.org/uniprot/P48357). Alternative nomenclature and protein sequence are given. LEPRb is the canonical sequence of LEPR. It is the most prevalent isoform, highly preserved among species, and has the longest amino acid sequence. LEPRb is the only isoform with clearly defined function and implications for body weight regulations.
Abbreviation: p., amino acid position in the protein.

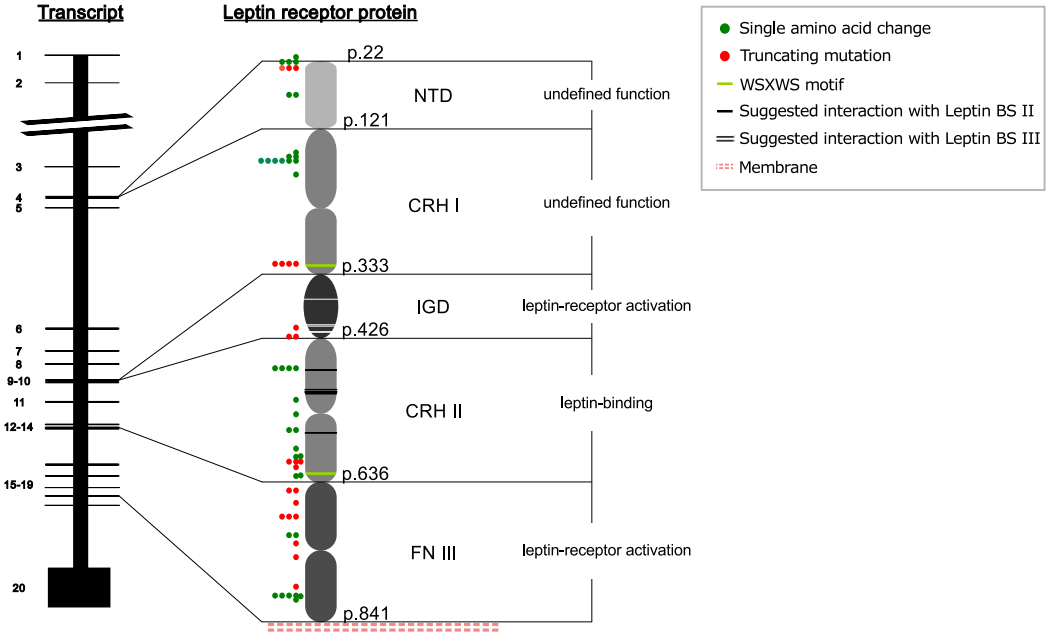

Figure 1. Simplified depiction of the *LEPR* gene and the extracellular domain of the mature LEPR protein and visualization of mutations in the human LEPR protein. Transmembrane and cytoplasmatic domains are not depicted, as no human mutations have been described in these domains. Every exon is numbered and assigned to the domain it encodes in the LEPR protein (the structure of the LEPR protein is based on the information of Peelman *et al.* [12]). The functional relevance of LEPR domains is given. LEP interacting sites are marked by white or black lines in the protein sketch and are positioned in CRHI/immunoglobulin-like domain (p.L372, A409, Y411, H419, H420) and CRHII (p.L471, Y472, F500, IFLL503-506, F563). Positions of WSXWS motifs are depicted as green lines: 319 to 323 and 622 to 626. Colored dots indicate and type affected protein position of human *LEPR* mutations; mutations result in single amino acid changes (green dots), or a truncated protein (red dots). BS, binding site; IGD, immunoglobulin-like domain; p., amino acid position in the protein.

The extracellular domain is divided into six subdomains (Fig. 1). The N-terminal domain is not required for LEP binding or signaling and is currently of undefined function. *In vitro* studies demonstrate that deletion of the subsequent cytokine receptor homology (CRH)I domain diminishes but does not abolish LEP signaling. In part, this appears to be caused by reduced cell surface expression [12–15]. LEP uses three binding sites denoted I to III to engage its receptor. Binding site I is of undefined function and its mutations affect receptor activation only marginally. Binding site II is required for receptor binding by interacting with the LEPR CRHII domain. Binding site III, in contrast, is responsible for receptor activation by interacting with the LEPR immunoglobulin-like domain (Fig. 1) [12, 16, 17]. LEPR activation furthermore requires the two fibronectin type III (FNIII) domains in membrane proximity [15, 16, 18]. The intracellular domain consists of a box 1 motif without kinase activity for binding of JAK2 and a variable number of tyrosine residues for mutual phosphorylation and activation of signaling cascades. LEPRb has three tyrosine residues (Y986, Y1079, Y1141) and is the only isoform that can induce STAT signaling [12, 19–21].

C. LEPR Signaling Cascade

In vitro studies show ligand-independent homodimerization of LEPR [22]. In the process of LEPR activation, LEP's binding site II interacts with the CRHII domain of the LEPR [12, 16, 23]. This causes a conformational change that favors formation of 2:2 or even higher complexes [13]. Lacking intrinsic kinase activity, LEPR signaling depends on JAK2 association to the receptor's box 1 motif [24]. Conformational changes of the LEP–LEPR complex result in

the activation of JAK2 by mutual phosphorylation of JAK2 and tyrosine residues of the intracellular receptor domain. Each of the phosphorylated tyrosine residues binds specific proteins that are crucial for further downstream signaling. For example, tyrosine residue Y986 accumulates SOCS3 and initiates a feedback loop mainly by inhibiting phosphorylation of the other tyrosine residues during prolonged signaling [25–27]. Y1079 plays a dominant role in activating STAT5, and Y1141 activates STAT3. STATs are subsequently phosphorylated by JAK2, dimerize, and translocate to the nucleus where they induce SOCS3 and POMC expression while repressing AgRP [15, 28, 29].

3. Identification of Mutations and Inheritance Patterns

We report six new patients with biallelic *LEPR* mutations and summarize 51 published cases. Reported mutations include 13 mutations that led to single amino acid changes, as well as 25 deletions, duplications, insertions, or nonsense mutations that were predicted to result in truncated LEPR proteins. Most mutations occurred in the FNIII and CRHII domains, whereas none was found in the intracellular domain. Detailed information is given in Table 2 and Table 3 and Fig. 1. Obesity caused by *LEPR* mutations was inherited in an autosomal-recessive pattern. There was no sex difference in distribution (27 females/30 males) (Supplemental Table 1). Ten subjects had a compound heterozygous mutations (Ulm_2 and Ulm_6) [30–34]. One patient had uniparental disomy, resulting in homoallelism in the *LEPR* gene inherited from the heterozygous father [35]. Consistent with the inheritance pattern, most patients were born to consanguineous parents, and obesity due to *LEPR* mutations aggregates in cultures with consanguineous marriages [36, 37].

4. Phenotype of Patients With Biallelic *LEPR* Mutations

A. Weight Gain and Hyperphagia

The most striking symptom of a loss of LEPR function was extreme and early-onset obesity. Birth weight was reported to be normal in all cases with regular pregnancies (data not shown), followed by extreme weight gain in the first months of life [6, 30, 38]. Mean body mass index (BMI, kg/m^2) for subjects <16 years of age was 40.3 (n = 31; 13 females, 18 males; mean age, 8 years) (Supplemental Table 1). Median body fat percentage and *z* score were slightly higher in females compared with males (Fig. 2A and 2B; Supplemental Table 1), but comparison is limited due to differences in age and methodology. In almost all cases, hyperphagia was described except in Gil_2.1, Gil-_2.2, and Han_2. Consistently, food impulsivity seems to be a common feature. Aggressive behavior was reported for Clem_1.1 to 1.3 and Ulm_3, 5, and 6 when food was withheld [6]. Lifestyle changes with increased physical activity and caloric restrictions mostly failed to reduce body weight in the long term [6, 35]. Clem_1.1 to 1.3 and Ulm_3 and 6 underwent a period of extreme calorie restriction without successful weight loss. Clem_1.1 to 1.3 had decelerated growth velocity as a result of caloric restriction [6].

B. Longitudinal Growth

Obese subjects often show accelerated growth with a decreased GH response and IGF-1 concentration. There is some discussion about the effects of impaired LEP signaling on longitudinal growth. Mouse models representing the counterpart of LEP (*ob/ob*; obese mouse model) and LEPR (*db/db*; diabetes mouse model) deficiency in humans show decreased linear growth and low GH concentrations [30, 35, 39–41] suggesting a role of LEP signaling for longitudinal growth. Reports on growth in humans with impaired LEPR function are heterogeneous. Farooqi *et al.* [30] and Kakar *et al.* [40] reported normal growth in childhood and diminished final height in adulthood due to a diminished

Table 2. Overview of Mutations in the Human *LEPR*

	Overview: LEPR Mutations						
First Author and Year of Publication	**Number of Cases (n)**	**Case ID**	**Patient Nationality**	**Mutation in the Coding DNA (c.)**	**Mutation in the Mature Protein (p.)**	**Affected Domain**	**Provided Functional Analysis**
Clement *et al.* 1998 [6]	3	Clem_1.1, Clem_1.2, Clem_1.3	Algerian	c.2597 + 1G>A	n.a.	FNIII	PCR and sequencing
Farooqi *et al.* 2007 [30]	2	Far_2.2, Far_2.1	Turkish	n.a.	11-bp del in codon 70	NTD	*In silico*
Farooqi *et al.* 2007 [30]	3	Far_4.3, Far_4.2, Far_4.1	Southern European	n.a.	p.W31*	NTD	n.a.
Farooqi *et al.* 2007 [30]	1	Far_3	Iranian	n.a.	66-bp del in codon 514	CRHII	*In silico*
Farooqi *et al.* 2007 [30]	1	Far_5	Turkish	c.1226C>A	p.A409E	IGD	*In vitro*
Farooqi *et al.* 2007 [30]	1	Far_6	Norwegian	n.a.	p.W664R	FNIII	*In vitro*
Farooqi *et al.* 2007; [30] Ulm	2	Far_7; Ulm_3	White (United Kingdom); German	c.2051A>C	p.H684P	FNIII	*In vitro*
Le Beyec *et al.* [35] 2013	1	Bey_1	French	c.1871dupA	p.N624Kfs*21	CRHII + FNIII	*In silico*
Kakar *et al.* 2013 [40]	5	Kak_1.1.1, Kak_1.1.2, Kak_1.2, Kak_1.3. Kak_1.4	Pakistani	c.1603 + 5G>C	p.R468Sfs*33	CRHII	*In silico*
Gill *et al.* 2013 [42]	2	Gil_2.1, Gil_2.2	Sudanese	c.479delA	p.H160Lfs*10	CRHI	*In silico*
Gill *et al.* 2013 [42]	1	Gil_1	Guinean	c.556delT	p.C186Afs*28	CRHI	*In silico*
Saeed *et al.* 2014 and 2015 [34, 38]	4	Sae_2, Sae2_3, Sae2_4, Sae2_5	Pakistani	c.2396-1G>T	n.a.	FNIII	*In silico*
Saeed *et al.* 2014 and 2015 [34, 38]	2	Sae_1, Sae2_6	Pakistani	c.1675G>A	p.W558*	CRHII	*In silico* Ilumina, Sanger
Huvenne *et al.* 2015 [32]	1	Huv_2	French	c.1810T>G	p.C604G	CRHII	*In silico*
Huvenne *et al.* 2015 [32]	1	Huv_3	Portuguese	c.2357T>C	p.L786P	FNIII	*In silico*
Huvenne *et al.* 2015 [32]	1	Huv_4	Turkish	c.2491G>A	p.H800_N831del	FNIII	*In silico*
Huvenne *et al.* 2015 [32]	5	Huv_5, Huv_6, Huv_7, Huv_8, Huv_9	French (Reunion Island)	Del exon 6–8	p.P166Cfs*7	CRHI	*In silico*, PCR
Ulm	1	Ulm_1	Turkish	Del exon 4–20	n.a.	CRHI-NTD	n.a.
Ulm	1	Ulm_4	German	Comp. het. c.2227 T>C and c.2598-3_2607delTAGAATGAAAAAG	Comp. het. p.S743P and p.Q865_K870	FNIII + CRHII	*In silico*
Ulm	1	Ulm_5	Turkish	p.N154Kfs*3	c.461dupA	CRHI	*In silico*
Ulm	1	Ulm_6	German	Comp. het. c.1874G>A and c.2051A>C	Comp. het. p.W625* and p.H684P	FNIII + CRHII	*In vitro* (p.H684P; see Farooqi *et al.* 2007 [30])
Farooqi *et al.* 2007 [30]	3	Far_1.3, Far_1.2, Far_1.1	Bangladeshi	n.a.	4-bp del in codon 22	NTD	*In silico*
Maezen *et al.* 2011 [37]	2	Maz_1, Maz_2	Egyptian	c.946C>A	p.P316T	CRHI	*In silico*
Andiran *et al.* 2011 [31]; Ulm	2	And_1, Ulm_2	Turkish	c.946C>A and c. n.a.	p.P316T and p.W646C (both homozygous)	CRHI + FNIII	*In silico*
Huvenne *et al.* 2015 [32]	1	Huv_10	French (Reunion Island)	Comp. het. c.1604–1G>A and del exon 6–8	Comp. het. p. n.a and p.P166Cfs*7	CRHII + CRHI	*In silico*
Hannema *et al.* 2016 [33]	1	Han_2	Dutch	c.1604–8A>G	K536Sfs*34 and p.V535Dfs*3[a]	CRHII	*In silico*
Vauthier *et al.* 2012 [39]	1	Vau_1	French	Del of *DNAJC6* and parts of *LEPR*	n.a.	NTD + CRII	PCR, MPLC
Huvenne *et al.* 2015 [32]	2	Huv_11.1, Huv_11.2	French	Comp. het. c.1264T>C and c.2131dup	Comp. het. p.Y422H and p.T711N fs*18	IGD + FNIII	*In silico*
Saeed *et al.* 2015 [34]	2	Sae2_2.1, Sae2_2.2	Pakistani	c.1810T>A	p.C604S	CRHII	*In silico*
Saeed *et al.* 2015 [34]	1	Sae2_1	Pakistani	Mutation not in transcript	n.a.	—	*In silico*
Hannema *et al.* 2016 [33]	1	Han_1	Dutch	Comp. het. c.1753–1dupG and c.2168C>T	Comp. het. p.M585Dfs*2 and p.S723F	CRHII	*In silico* Ilumina, Sanger
Farooqi *et al.* 2007 [30]	1	Far_8	White (United Kingdom)	Comp. het. c. n.a. and c.1835G>A	Comp. het. 1 bp del in codon 15 and p.R612H	NTD + CRHII	*In vitro* (p.R612H)

Included are number of cases, case ID and nationality, location of the mutation in the LEPR protein, affected domain, and provided information about functional analysis. Estimation of the functional relevance of the respective mutation was made based on predefined criteria. Criteria for functional relevance were (1) highly suspicious BMI, (2) hypogonadotropic hypogonadism, (3) consanguineous parents, (4) highly suspicious variant, and (5) conclusive functional analysis. Conclusions on functional relevance are based on the number of fulfilled criteria: "High" indicates high evidence for complete loss of LEPR function (three to five criteria fulfilled); "Probably" indicates that the mutation is probably damaging (two to three criteria); "Low" indicates low evidence for functional relevance, with *in vitro* analyses necessary to exclude residual function of LEPR (two or fewer criteria).

Abbreviations: c., cDNA position in the gene; comp. het., compound heterozygous; del, deletion; fs, frameshift; HH, hypogonadotropic hypogonadism; MPLC, medium pressure liquid chromatography, n.a., no information available; p., amino acid position in the protein; Rf, residual function; *, premature stop codon.

[a]Published as corresponding to p.K597Sfs*34 and p.V596Dfs*3 in the original paper. Based on the experimentally validated changes in the RNA, we assume the correct mutations to be p.K536Sfs*34 and p.V535Dfs*3.

Table 3. Estimation of Functional Relevance of Mutations in the Human *LEPR*

Overview: LEPR Mutations	Estimation of Functional Relevance					
	Criteria for Functional Relevance					
Case ID	Suspicious BMI	HH	Consanguineous Parents	Suspicious Variant	Conclusive Functional Analysis	Evidence for Functional Relevance
Clem_1.1, Clem_1.2, Clem_1.3	X	X	X	X	X	High
Far_2.2, Far_2.1	X	X	X	X		High
Far_4.3, Far_4.2, Far_4.1	X	X	X	X		High
Far_3	X		X	X		High
Far_5	X		X	X	X	High
Far_6	X		X	X	X	High
Far_7; Ulm_3	X				X	High
Bey_1	X	X	X	X		High
Kak_1.1.1, Kak_1.1.2, Kak_1.2, Kak_1.3. Kak_1.4	X (not for Kak_1.1.1)		X	X		High
Gil_2.1, Gil_2.2	X	X	X	X		High
Gil_1		X	X	X		High
Sae_2, Sae2_3, Sae2_4, Sae2_5	X		X	X		High
Sae_1, Sae2_6	X		X	X	X	High
Huv_2	X		X	X		High
Huv_3	X	X	X			High
Huv_4	X		X	X		High
Huv_5, Huv_6, Huv_7, Huv_8, Huv_9	X	X			X	High
Ulm_1	X		X	X		High
Ulm_4	X	X		X		High
Ulm_5	X		X	X		High
Ulm_6	X		X		X	High
Far_1.3, Far_1.2, Far_1.1	X (only Far_1.1)		X	X		Probably
Maz_1, Maz_2	X	X	X			Probably
And_1, Ulm_2	X	X	X			Probably
Huv_10	X			X		Probably
Han_2		X		X		Probably
Vau_1						Low
Huv_11.1, Huv_11.2	X					Low (for p.Y422H)
Sae2_2.1, Sae2_2.2			X			Low
Sae2_1	X		X			Low
Han_1						Low (for p.S723F)
Far_8						Rf

pubertal growth spurt. In contrast, Gil_2.1, Sae_1, Ulm_2, Ulm_6, and Clem1.1 to 1.3 showed diminished growth throughout childhood [6, 38, 42] and Maz_1, And_1, Gil_1, Sae_2, and Ulm_3 presented with accelerated growth (Supplemental Table 1) [31, 37, 38, 42].

Likewise, serum concentrations of GH and IGF-1 were heterogeneous. Clément *et al.* [6], Le Beyec *et al.* [35], and Huvenne *et al.* [32] (Huv_2, 6, 8, and 9) reported low GH values. Clément *et al.* [6] and Andiran *et al.* [31] found low IGF-1 concentrations. Conversely, Farooqi *et al.* [30] found normal IGF-1 and GH concentrations. Vau_1, Bey_1, Han_1 and 2, and Ulm_1 to 6 had normal IGF-1 values (Supplemental Table 1). Data for GH serum concentration were scarce and are therefore not listed in a table. Taken together, these data do not allow for a conclusion as to whether LEP signaling plays a role in longitudinal growth.

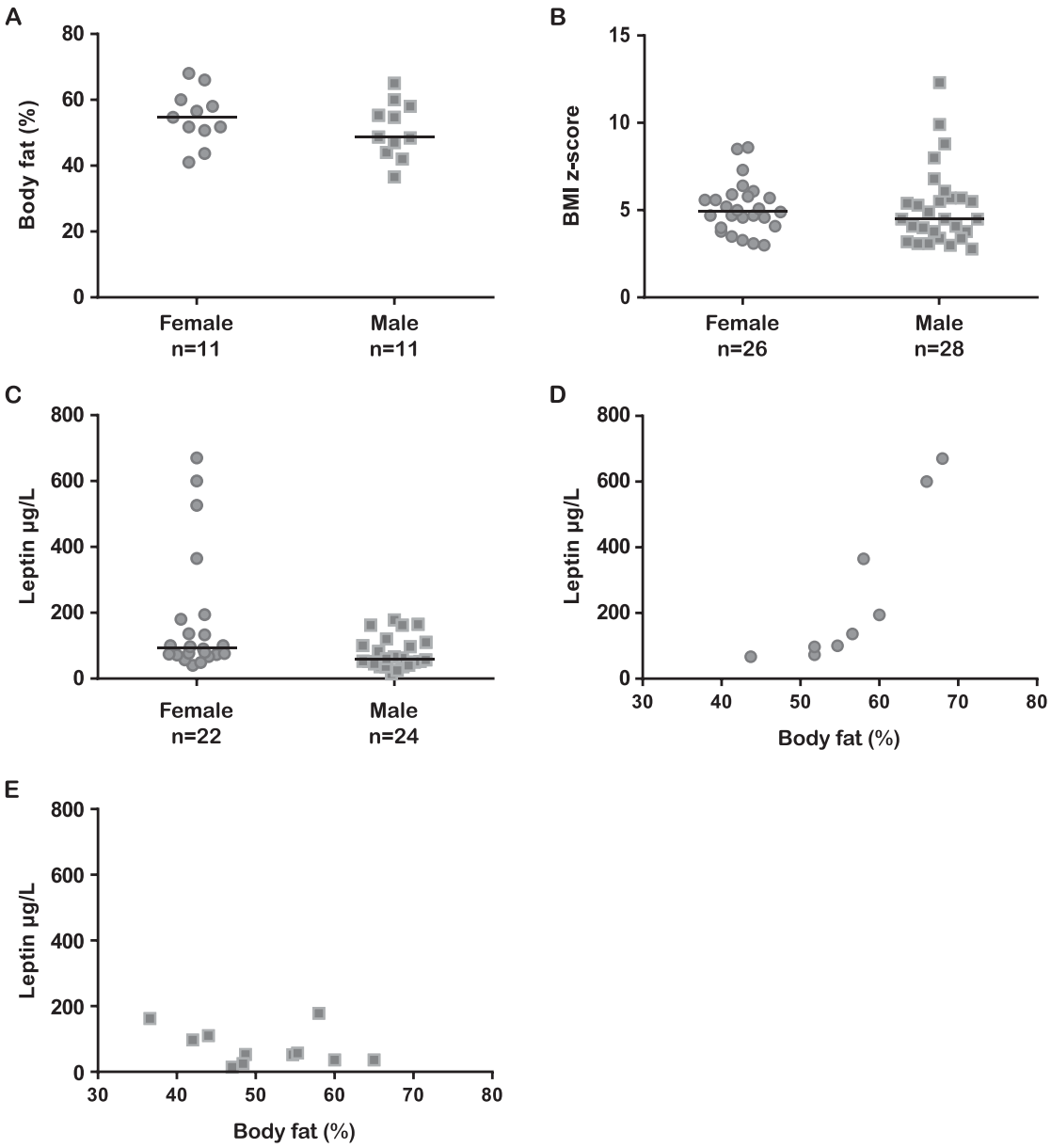

Figure 2. Body fat percentages, BMI *z* scores, and serum LEP concentrations in patients with biallelic *LEPR* mutations. (A) Body fat percentage by sex (mean age females, 18.2 y; mean age males, 10.1 y; median females, 54.7%; median males 48.7%). (B) BMI *z* score by sex (mean age females, 15.8 y; mean age males, 7.8 y; median females, 4.95; median males, 4.5). (C) Serum LEP concentrations by sex (mean age females, 17.2 y; mean age males, 8.0 y; median females, 93.5 µg/L; median males, 58.4 µg/L). (D) Correlation of body fat percentage and serum LEP concentrations in females (mean age, 19.4 y; n = 9). (E) Correlation of body fat percentage and serum LEP concentrations in males (mean age, 10.1 y; n = 11).

C. Pubertal Development and Fertility

Comparable to the findings in *ob/ob* and *db/db* mice and individuals with LEP deficiency, subjects with impaired LEPR function showed disrupted pubertal development [41, 43, 44]. In healthy individuals, circulating LEP concentrations increase before the onset of puberty [45], and some studies indicate that LEP affects GnRH neuron activity and GnRH secretion by crosstalk with kisspeptin [46]. Accordingly, pulsatile secretion of GnRH (the trigger of

increased LH and FSH secretion and gonadal activation) is disturbed in individuals with LEP signaling defects [47, 48].

Von Schnurbein *et al.* [43] showed that regular subcutaneous injections of a LEP substitute (metreleptin; a recombinant analog of LEP) induced and normalized pulsatile gonadotropin secretion in a LEP-deficient adolescent girl. LEP substitution cannot be considered as a treatment option for patients with LEPR dysfunction. Interestingly, for LEPR-deficient patients >20 years of age, normalization of sex hormones and delayed menses have been reported (see Far_4.1 to 4.3 in Supplemental Tables 1 and 2). This normalization seems to be favored by strict weight control (see Clem_1.1, Bey_1, Huv_5, Ulm_4, and Ulm_6; for details, see "Treatment Outcomes With Bariatric Surgery" below). For instance, Clem_1.1 showed no pubertal development when she was 13.5 years old [6]. At the age of 18 years, Clem_1.1 underwent bariatric surgery (for details, see "Treatment Outcomes With Bariatric Surgery" below) and lost 50 kg in 6 months. After surgery, sex hormones normalized and she was able to conceive. The mother gained 50 kg during pregnancy without any features of gestational diabetes mellitus. The fetus developed regularly [49].

Huv_5, Huv_11.1, and Huv_11.2 showed no signs of hypogonadotropic hypogonadism [32]. It would be interesting to assess whether there was a residual STAT5 phosphorylation by the LEPR in these cases. In mouse models, an exchange of tyrosine residue 1138 to serine induced impaired STAT3 signaling with residual STAT5 function [50]. This resulted in an obese phenotype with normal pubertal development and fertility. Similar mutations with residual STAT5 activity may also occur in humans.

D. Metabolic Features

LEP has been shown to have a direct effect on insulin sensitivity and was suggested as a novel therapeutic agent in metabolic disorders, including insulin resistance and type 2 diabetes [51]. In the *db/db* mouse model, the incidences of type 2 diabetes and hepatic steatosis are increased [52, 53]. Summarizing the published data in humans, four individuals (Far_4.2, Far-_4.1, Gil_1, and Huv_11.1) suffered from type 2 diabetes. Moreover, 17 patients with elevated insulin concentrations were reported. Of these, five were <10 years of age (Supplemental Table 3). This suggests that LEPR-deficient subjects may be at risk for early-onset insulin resistance and diabetes [54].

In 2013, von Schnurbein *et al.* [55] reported hepatic steatosis in a LEP-deficient patient, a feature that is also common in lipodystrophic subjects who have very low LEP levels. Findings indicative of hepatic steatosis seen upon ultrasound examination were reported for Gil_1 and Ulm_1, 2, 4, and 6 [42]. Fasting triglyceride concentrations were reported in few patients and were increased in Han_1 and Ulm_1. Maz_1 and 2 had triglyceride levels within the reference range.

E. Thyroid Function

Several *in vitro* and *in vivo* studies propose an involvement of LEP in thyroid function. A decrease in LEP concentration diminishes TRH expression in the hypothalamic paraventricular nucleus, resulting in decreased TSH and thyroid hormone production. LEP may affect TRH expression directly by activating LEPRb on TRH neurons [56], or indirectly by enhancing α-melanocyte–stimulating hormone and suppressing AgRP production and thus disinhibiting TRH secretion [57].

In concordance with this, Clément *et al.* [6] and Montague *et al.* [58] propose a role for LEP in controlling thyroid function in humans. Indeed, there are few reports on impaired function of the thyroid axis in patients with LEPR deficiency (Clem_1.1 to 1.3 and Huv_9, hypothalamic hypothyroidism; Bey_1, low T4 normal basal TSH; Ulm_2, elevated basal TSH; Ulm_4, reduced free thyroxin) (Supplemental Table 3).

F. Immune Function

The effect of LEP or LEPR deficiency in *ob/ob* or *db/db* mouse models is well studied. The lack of LEP leads to an abnormal cytokine secretion and thymic hypotrophy that can be

remedied by metreleptin administration in *ob/ob* mice [59]. Patients with LEP deficiency due to a mutation in the *LEP* gene may also show increased susceptibility to infections and show a decreased ratio of CD4/CD8 T-lymphocytes in these patients [60, 61]. Likewise, evidence for immune dysfunction has been described for subjects with LEPR dysfunction. For example, Far_1-8, Maz-_1 and 2, Ulm_6, and Sae_1 and 2 suffered from pulmonary infections at an early age and had frequent episodes of diarrhea. Farooqi *et al.* [30] reported reduced T-cell number with low proliferation rates and compensatory increased in B-cell counts [30]. Vauthier *et al.* [39] described a reduced CD4/CD8 T-cell ratio (low CD4 and high CD8), but more natural killer cells in Vau_1.

G. LEP Serum Concentrations

We found a wide range between the reported LEP serum concentrations in subjects with LEPR deficiency (Supplemental Table 1). This might in part be attributable to assay variability. Additionally, truncating LEPR mutations leading to a soluble LEPR (sLEPR)–like product (as is the case for Clem_1.1-1.3) result in highly elevated serum LEP concentrations (measured as bound or total LEP) [62]. Most authors have related the measured LEP concentrations to a BMI- or fat percentage–matched reference population. Thereby, normal serum LEP concentrations were reported by Farooqi *et al.* [30] in 2007, Saeed *et al.* [38] in 2014, and Hannema *et al.* [33] in 2016. Elevated values compared with BMI-matched individuals were reported by Le Beyec *et al.* [35] in 2013, Gill *et al.* [42] in 2014, Huvenne *et al.* [32] in 2015, and Saeed *et al.* [34] in 2015. Using the published values, LEP concentrations and body fat percentage seem to correlate stronger in females than in males. However, this comparison is limited by the large age difference between the groups (Fig. 2D and 2E). Standardized analytical methods are needed for qualitative statements about LEP concentration in LEPR-deficient subjects.

H. Treatment Outcomes With Bariatric Surgery

Reports about the success of bariatric surgery in subjects with LEPR defects gave conflicting results [32, 35, 49]. We are aware of six subjects with LEPR defects who have undergone bariatric surgery (Table 4). Clem_1.1, Huv_2, and Ulm_4 were not able to maintain reduced body weight. Clem_1.1 regained weight after the above-mentioned unexpected pregnancy (see "*Pubertal Development and Fertility*" above and Supplemental Table 1) [6, 32, 49]. Ulm_4 had her menarche with six regular cycles during the phase of weight reduction. After weight regain, menses cheesed again.

Bey_1 and Ulm_6 successfully managed to reduce and maintain body weight after surgery, whereby sex hormones normalized [35]. There is no clear evidence whether this was due to the surgery or his advanced age. However, for Ulm_6, malformed sperms, reduced sperm number, and sperm velocity were reported. There are no long-term reports about Huv_3 (Table 4).

In summary, bariatric surgery seems less effective in the female subjects, especially in the long term. The normalization of sex hormones after surgery is in line with outcomes in obese patients without known underlying genetic cause and suggests a mechanism independent of LEP signaling [63, 64].

I. Treatment Outcomes With MC4R Agonist Setmelanotide

Causal treatment of patients with *LEPR* mutations is not yet available. Setmelanotide, an MC4R agonist, is an interesting candidate. Setmelanotide has been shown to be extremely effective in reducing appetite and body weight in patients with POMC deficiency [65]. It is physiologically plausible that patients with LEPR defects will show a similar response. Preliminary results of a phase 3 clinical trial show substantial weight loss (−19.6%) in one patient with LEPR deficiency after 22 weeks of setmelanotide treatment without reported adverse events [66].

Table 4. LEPR-Deficient Patients Who Underwent Bariatric Surgery

Patient ID	Age at Surgery (y)	Sex	Type of Intervention	Achieved Weight Loss (kg)	Complications/Weight Regain
Clem_1.1	16	F	Abdominoplasty	n.a.	n.a.
	24		Gastric bypass	50	Weight regain after pregnancy (+50 kg)
Bey_1	16	M	Gastric banding	46	Gastric band slippage, weight regain
	18		Gastroplasty	40	—
Huv_2	n.a.	F	Gastric bypass	45	Weight regain (+34 kg)
Huv_3	n.a.	M	Gastroplasty	−44% of body weight	No follow-up data
Ulm_4	18	F	Sleeve gastrectomy	30	Weight regain (+19 kg)
Ulm_6	14	M	Gastric banding	47	Gastric band slippage, weight regain (+10 kg)

We report age at surgery, type of surgery, weight loss in kilograms, reported complications, and the weight regain surgery where information is available.
Abbreviations: F, female; M, male; n.a., no information available.

J. Psychomotor Development and Social Behavior

Gil_1, Vau_1, Ulm_3, and Ulm_5 have been reported to be developmentally delayed. However, in the case of Vau_1, this could also be due to a concomitant auxillin-1 deficiency caused by the reported 80-kb deletion, which also affects the *DNAJC6* gene [39, 67]. Ulm_3 was diagnosed with hearing impairment and delays in psychomotor, statomotoric, and speech development. These delays occurred in the setting of a complicated pregnancy with gestational diabetes. He was born at 30 weeks with a body weight of 1.6 kg and bilateral hearing impairment. Ulm_5 was diagnosed with cognitive delays. Clément *et al.* [6] described impulsive behavior, emotional lability, and social disability in Clem_1.1-1.3. In concordance with these findings, we observed defiant behavior and various degrees of psychological abnormalities, including addictive behavior, in the patients Ulm_1 to 6.

5. Functional Analysis of Mutations in Human LEPR

Summarizing clinical data, severe early-onset obesity, hyperphagia, and hypogonadotropic hypogonadism are cardinal features of a complete loss of LEPR function (Supplemental Table 1). In contrast, symptoms such as recurring infections, altered growth, developmental delay, and metabolic disorders showed variable manifestations. Differences in the clinical appearance may be related to residual LEPR activation and may depend on the character and location of the mutation. We therefore investigated whether there is any relationship between genotype and phenotype. For instance, variants affecting solely the N-terminal domain and CRHI domains are thought to be less crucial for receptor function [12]. Furthermore, compound heterozygous variants could have partially preserved function when different domains are affected. Functional *in silico* analyses were available for 35 mutations (Table 2 and Table 3). Functional *in vitro* data were available for four mutations. Farooqi *et al.* [30] confirmed the functional impairment of p.A409E, p.W664R, and p.H684P and reported residual STAT3 phosphorylation in p.R612H. According to our own investigations of the literature, evaluation of the clinical appearance of the described subjects, as well as provided functional analysis, we further expect residual LEPR function in Vau_1, Huv_11.1, Huv_11.2, Sae2_2.1, Sae2_2.2, Sae2_1, and Han_1 (Table 2 and Table 3). These suggestions need to be verified by functional *in vitro* analysis. Based on the available data, we did not identify an obvious genotype–phenotype relationship, which might be partly due to incomplete information in the literature.

In another approach we categorized mutations in the human LEPR based on the hypothesized impact of respective mutation on the protein product, should translation still occur: (1) truncated protein not able to bind LEP; (2) sLEPR-like molecule; (3) membrane-bound

LEPR with no ability to bind LEP; and (4) membrane-bound LEPR with no ability to elicit cellular signaling (Supplemental Table 4). We suggest that mutations belonging to category 2 will result in a sLEPR-like protein resulting in elevated total and bound LEP concentration in the bloodstream, as reported by Lahlou *et al.* [62]. Furthermore, mutations belonging to categories 3 and 4 might be interesting candidates for further investigations of genotype–phenotype correlations and functional analysis.

6. Conclusion

We present a structured and comprehensive analysis of a large patient cohort with biallelic *LEPR* mutations comprising all published cases to date. In total, 57 subjects including six yet unpublished patients from our outpatient clinic with 38 distinct *LEPR* mutations were identified. Most mutations occurred in the FNIII and CRHII domains.

Functional aspects of the mutations were available from *in silico* or *in vitro* analyses for 29 mutations, showing residual STAT3 phosphorylation in one mutation. Considering clinical phenotype and character of the respective mutations, we suspect residual function in five additional mutations.

LEPR deficiency causes severe early-onset obesity, hyperphagia, and hypogonadotropic hypogonadism. Association with immune dysfunction, growth restriction, psychomotor delays, and metabolic disorders is variable without any identified genotype–phenotype relationship. Biallelic LEPR mutations should be considered a rare and serious disease associated with severe social and psychological burdens for patients and their families. We propose that findings of *LEPR* mutations in patients should be harmonized with associated phenotypic characteristics in a structured and comprehensive way by establishing an international registry for this rare disease.

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