# Functionality and Phenotypic Characteristics of Human Leptin Receptor Mutations

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## Conclusion

These results represent a structured and comprehensive analysis of a large patient cohort with mutations in the *LEPR*. LEPR-deficiency is a serious disease characterized by severe early-onset obesity, hyperphagia and hypogonadotropic hypogonadism. Further, there is a heterogeneous disease spectrum concerning e.g. infections, growth, developmental delay and type 2 diabetes. Till now, we were not able to reveal a genotype-phenotype-correlation. Based on provided information about functional analysis, mutation size, and location, as well as phenotypic characteristics of affected patients we suggest residual LEPR function in 6 Mutations. Future *in vitro* analysis should confirm this findings.

## Objective

In this project we aimed to summarize published and unpublished functional and phenotypic data on mutations in the human leptin receptor (*LEPR*) gene causing a rare form of severe early-onset obesity. Further, we estimated the functional relevance of described mutations in the human *LEPR* and we investigated a possible genotype-phenotype-correlation.

## Methods

Literature research was performed using PubMed and OMIM. Additional data was obtained from 6 subjects of our outpatient clinic not reported so far. Functional relevance of mutations was estimated based on reported functional analysis, mutation size, and location, as well as phenotypic characteristics of affected patients.

## Results

**Table 1a:** Overview of mutations in the human *LEPR* (case ID, first author and year of the publication, number of cases, location of the mutation in the LEPR protein, and affected domain). **Table 1b:** Estimations of the functional relevance of the respective mutation were made based on predefined criteria:(1) highly suspicious BMI; (2) hypogonadotropic hypogonadism (HH); (3) consanguineous parents, (4) highly suspicious variant; (5) conclusive functional analysis. Conclusions on functional relevance are based on the number of fulfilled criteria: "*high*"= high evidence for complete loss of LEPR function (3 to 5 criteria fulfilled); "*probably*"= mutation is probably damaging (2 to 3 criteria); "*low*"= low evidence for functional relevance, in vitro analyses are necessary to exclude residual function of LEPR (0 to 2 criteria).

Table 1a Overview-LEPR mutations

| ID | First author and year of the publication | Cases (n) | Mutation in the mature protein | Affected domain |
|---|---|---|---|---|
| 1 | Clement et al. 1998 | 3 | n.a. | FNIII |
| 2 | Farooqi et al. 2007 | 2 | 11-bp del in codon 70 | NTD |
| 3 | Farooqi et al. 2007 | 3 | p.W31* | NTD |
| 4 | Farooqi et al. 2007 | 1 | 66-bp del in codon 514 | CRHII |
| 5 | Farooqi et al. 2007 | 1 | p.A409E | IGD |
| 6 | Farooqi et al. 2007 | 1 | p.W664R | FNIII |
| 7 | Farooqi et al. 2007; Ulm | 2 | p.H684P | FNIII |
| 8 | Le Beyec et al. 2012 | 1 | p.N624Kfs*21 | CRHII+FNIII |
| 9 | Kakar et al. 2013 | 5 | p.R468Sfs*33 | CRHII |
| 10 | Gill et al. 2013 | 2 | p.H160Lfs*10 | CRHI |
| 11 | Gill et al. 2013 | 1 | p.C186Afs*28 | CRHI |
| 12 | Saeed et al. 2014&2015 | 3 | n.a. | FNIII |
| 13 | Saeed 2014&2015 | 2 | p.W558* | CRHII |
| 14 | Huvenne et al. 2015 | 1 | p.C604G | CRHII |
| 15 | Huvenne et al. 2015 | 1 | p.L786P | FNIII |
| 16 | Huvenne et al. 2015 | 1 | p.H800_N831del | FNIII |
| 17 | Huvenne et al. 2015 | 5 | p.P166Cfs*7 | CRHI |
| 18 | Ulm | 1 | n.a. | CRHI-NTD |
| 19 | Ulm | 1 | comp. het. p.S743P & p.Q865_K870 | FNIII+CRHII |
| 20 | Ulm | 1 | c.461dupA | CRHI |
| 21 | Ulm | 1 | comp. het. p.W625* & p.H684P | FNIII+CRHII |
| 22 | Farooqi et al. 2007 | 3 | 4-bp del in codon 22 | NTD |
| 23 | Maezen et al. 2011 | 2 | p.P316T | CRH I |
| 24 | Andiran et al 2011 & Ulm | 2 | p.P316T & p.W646C (both homozygous) | CRHI+FNIII |
| 25 | Huvenne et al. 2015 | 1 | comp het. p. n.a & p.P166Cfs*7 | CRHII+CRHI |
| 26 | Hannema et al. 2016 | 1 | K536Sfs*34 & p.V535Dfs*3[1] | CRHII |
| 27 | Vauthier et al. 2012 | 1 | n.a. | NTD+CRII |
| 28 | Huvenne et al. 2015 | 2 | comp.het. p.Y422H & p.T711N fs*18 | IGD+FNIII |
| 29 | Saeed et al. 2015 | 2 | p.C604S | CRHII |
| 30 | Saeed et al. 2015 | 1 | n.a. | - |
| 31 | Hannema et al. 2016 | 1 | comp. het. p.M585Dfs*2 & p.S723F | CRHII |
| 32 | Farooqi et al. 2007 | 1 | comp. het. 1 bp del in codon 15 & p.R612H | NTD+CRHII |

Table 1b Estimation of functional relevance

| ID | Provided functional analysis | Criteria for functional relevance | | | | | Evidence for functional relevance |
|---|---|---|---|---|---|---|---|
| | | Suspicious BMI | HH | Consanguineous parents | Suspicious variant | Conclusive functional analysis | |
| 1 | PCR and sequencing | X | X | X | X | X | high |
| 2 | in silico | X | X | X | X | | high |
| 3 | n.a. | X | X | X | X | | high |
| 4 | in silico | X | | X | X | | high |
| 5 | in vitro | X | | X | X | X | high |
| 6 | in vitro | X | | X | X | X | high |
| 7 | in vitro | X | | | | X | high |
| 8 | in silico | X | X | X | X | | high |
| 9 | in silico | X | | X | X | | high |
| 10 | in silico | X | X | X | X | | high |
| 11 | in silico | | X | X | X | | high |
| 12 | in silico | X | | X | X | | high |
| 13 | in silico, Ilumina, Sanger | X | | X | X | X | high |
| 14 | in silico | X | | X | X | | high |
| 15 | in silico | X | X | X | | | high |
| 16 | in silico | X | | X | X | | high |
| 17 | in silico, PCR | X | X | | | X | high |
| 18 | n.a. | X | | X | X | | high |
| 19 | in silico | X | X | | X | | high |
| 20 | in silico | X | | X | X | | high |
| 21 | in vitro (for p.H684P) | X | | X | | X | high |
| 22 | in silico | X | | X | X | | probably |
| 23 | in silico | X | X | X | | | probably |
| 24 | in silico | X | X | X | | | probably |
| 25 | in silico | X | | | X | | probably |
| 26 | in silico | | X | | X | | probably |
| 27 | PCR, MPLC | | | | | | low |
| 28 | in silico | X | | | | | low (for p.Y422H) |
| 29 | in silico | | | X | | | low |
| 30 | in silico | X | | X | | | low |
| 31 | in silico, Ilumina, Sanger | | | | | | low (for p.S723F) |
| 32 | in vitro (p.R612H) | | | | | | Rf |

In total 57 subjects with 38 distinct mutations in the *LEPR* were identified. From 38 mutations,13 led to a single amino acid change. 25 deletions, duplications, insertions or nonsense mutations resulted in truncated LEPR proteins (Table 1a & Figure 1). *In silico* analysis were reported for 23 mutations. Functional data from *in vitro* experiments were available for 4 mutations, showing residual function in one. Considering clinical phenotype and character of respective mutations, we suspect residual function in 5 additional mutations (Table 1b).

Summarizing clinical data, we found severe early-onset obesity, hyperphagia, and hypogonadotropic hypogonadism as cardinal features of a complete loss of LEPR function. Other disease e.g. metabolic disorders and recurring infections were more variable in manifestation.

Median body fat percentage and z-score were slightly higher in female compared to male (Figure 2 A & B), but comparison is limited due to differences in age and methodology.

We found a wide range between the reported LEP serum concentrations in subjects with LEPR deficiency. This might in part be attributable to assay variability. In addition, truncating LEPR mutations leading to a soluble LEPR like product (as it is the case for ID 1) result in highly elevated serum leptin concentrations (measured as bound or total leptin) (8).

Using the published values, LEP concentrations and body fat percentage seem to correlate stronger in females than in males. Also, this comparison is limited by the large age difference between the groups (Figure 2D and E). Standardized analytical methods are needed for qualitative statements about LEP concentration in LEPR deficient subjects

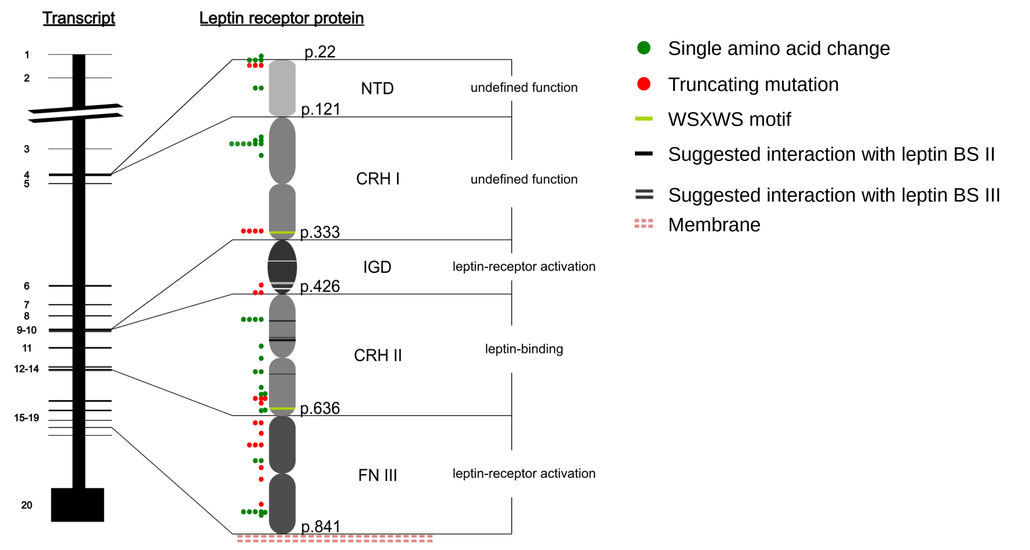


**Figure 1:** Simplified depiction of the *LEPR* transcript, the extracellular domain of the mature LEPR protein and visualization of mutations in the human LEPR protein. Every exon is numbered and assigned to the domain it encodes in the LEPR protein (the structure of the LEPR protein is based on the information of Peelman et al (1). Functional relevance of LEPR domains is given. Leptin interacting sites are marked by white or black lines in the protein sketch and are positioned in IGD (p.L372, A409, Y411, H419, H420) and CRHII (p.L471, Y472, F500, IFLL503-506, F563)(1-7). Positions of WSXWS motifs: 319-323 and 622 – 626. Abbreviations see below.

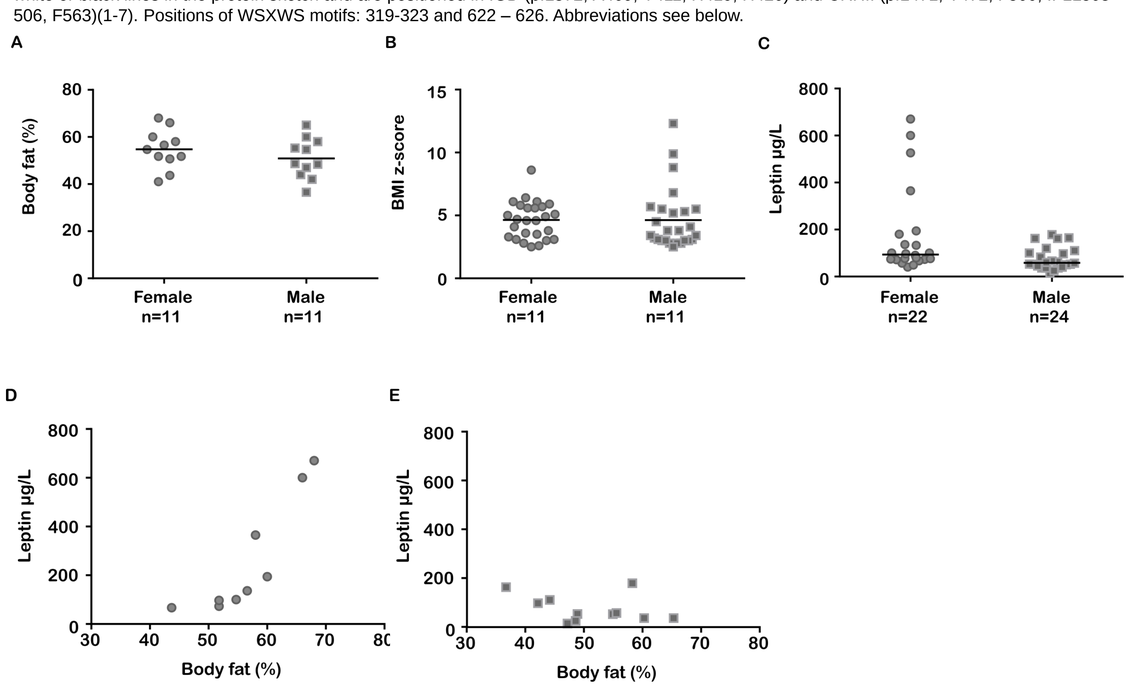


**Figure 2:** Body fat percentages, BMI z-scores and serum leptin concentrations in patients with biallelic *LEPR* mutations.
A: Body fat percentage by gender (mean age females=18.2y; mean age males=10.1y; median body fat: females=54.7%; males=48.7%); B: BMI z-score by gender (mean age females=15.8y; mean age males=7.8y; median z-score females=4.65; males=3.6); C: Serum leptin concentrations by gender (mean age females=17.2y; mean age males=8.0y; median leptin concentrations females=93.5µg/L; males=58.4µg/L); D: Correlation of body fat percentage and serum leptin concentrations in females (mean age=19.4y; n=9); E: Correlation of body fat percentage and serum leptin concentrations in males (mean age=10.1y; n=11)

**Abbrevations and Notes**: NTD= N-terminal domain, CRH= cytokine receptor homology, IGD= immunoglobulin-like domain, FN= fibronectin type bp= base pair, comp. het.= compound heterozygous, del= deletion, fs= frameshift, MPLC= Medium Pressure Liquid Chromatography, n.a.= no information available; c.= cDNA position in the gene, p.= amino acid position in the protein, PCR= polymerase chain reaction, Rf= residual function, *= premature stop codon. Notes: 1, 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*3.0

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**Disclosure Statement**
The authors have nothing to disclose