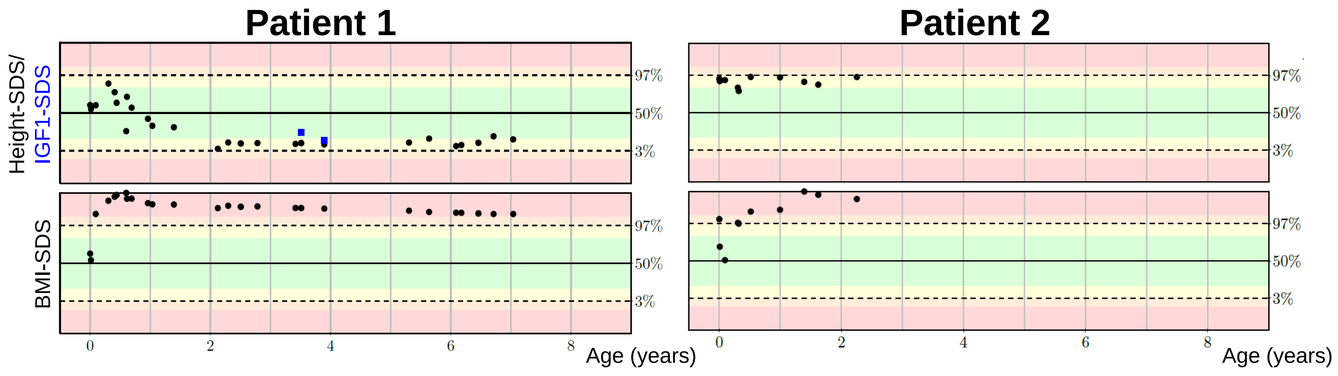
Functional characterization of novel and known genetic variants in the leptin receptor (*LEPR*) gene of two patients with morbid obesity

Franziska Voigtmann[1,3], Robert Stein[1], Philipp Wolf[2], Kathrin Landgraf[1,3], Rami Abou Jamra[4], Wieland Kiess[1], Annette G. Beck-Sickinger[2], Antje Körner[1,3]

[1] Center for Pediatric Research Leipzig (CPL), University Hospital for Children & Adolescents, Germany [2] Institute of Biochemistry, Faculty of Bioscience, Pharmacy and Psychology, Leipzig University, Germany [3] Integrated Research and Treatment Center (IFB) Adiposity Diseases, Leipzig University, Germany [4] Institute of Human Genetics, University Hospital Leipzig, Germany

Background & Objectives

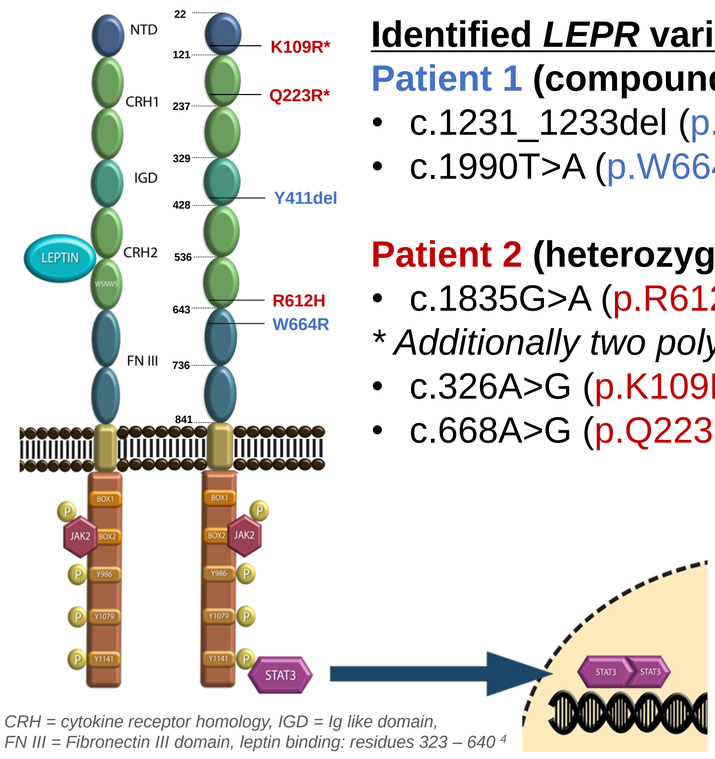


	Patient 1	Patient 2
Birth		
Gestational age (weeks)	40	41 + 3
Gestational diabetes	No	No
Length (cm) / Percentile	52 / P65	55 / P91
Weight (g) / Percentile	3560 / P71	4560 / P99
BMI-SDS / Percentile	0.51 / P69	1.64 / P95
Clinical and laboratory findings on first presentation		
Age (years)	2.3	1.6
Hyperphagia	Yes	Yes
Frequency of infections	Normal	Normal
Hypothyroidism	Yes	No
Family history		
Ethnic origin	Russian (both parents)	Turkish (paternal)
Consanguinity	No	No
Obese family members	Yes	No

Whole exome sequencing of two patients with early-onset morbid obesity and hyperphagia revealed novel and known variants in the *LEPR* gene.

The leptin receptor pathway comprises

- extracellular leptin binding
- receptor dimerization
- STAT3 phosphorylation
- induction of a satiety signal [1,2]



CRH = cytokine receptor homology, IGD = Ig like domain, FN III = Fibronectin III domain, leptin binding: residues 323 – 640 [4]

Identified *LEPR* variants

Patient 1 (compound heterozygous):

- c.1231_1233del (p.Y411del) → novel
- c.1990T>A (p.W664R) → known [3]

Patient 2 (heterozygous):

- c.1835G>A (p.R612H) → known [3]

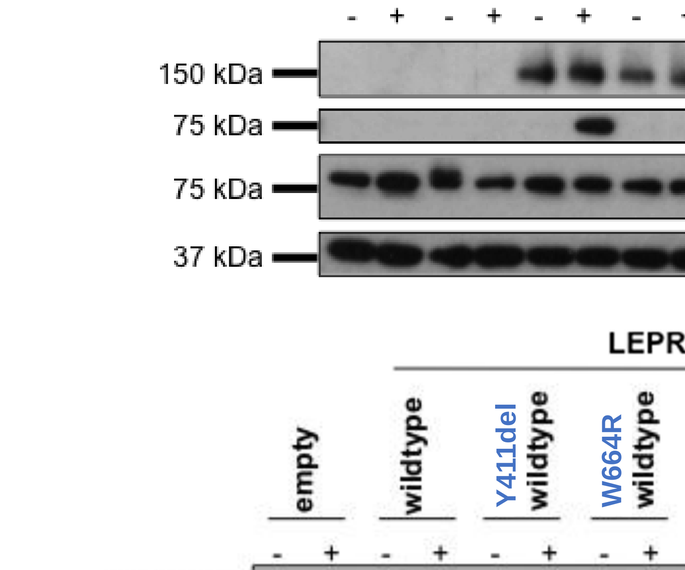
** Additionally two polymorphisms:*

- c.326A>G (p.K109R)
- c.668A>G (p.Q223R)

Aim: To characterize the functional impact of the identified variants on a cellular level using transfected HEK 293 cells.

Results

1. Reduced expression of *LEPR* variants

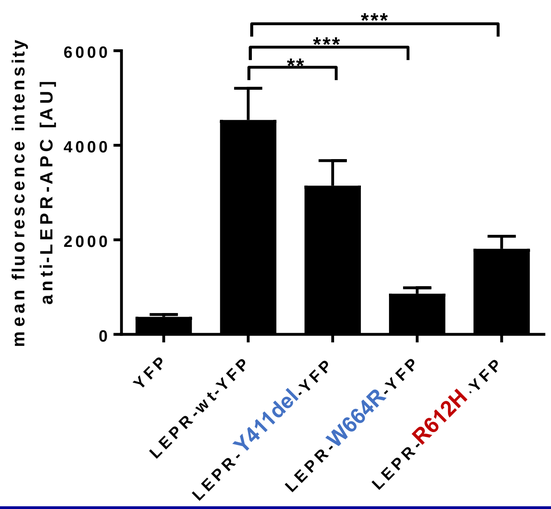


Quantitative PCR of transfected HEK 293 cells showed mildly diminished expression of variant *LEPR* constructs.

2. Homozygous and heterozygous expression of *LEPR* variants impairs STAT3 phosphorylation

Immunoblotting of HEK 293 cells, transfected with myc-tagged *LEPR* constructs. STAT3 phosphorylation upon leptin stimulation was absent with variant Y411del and W664R, but not with R612H. Similar results were obtained when mimicking (compound) heterozygous expression.

3. Diminished cell surface expression of *LEPR* variants

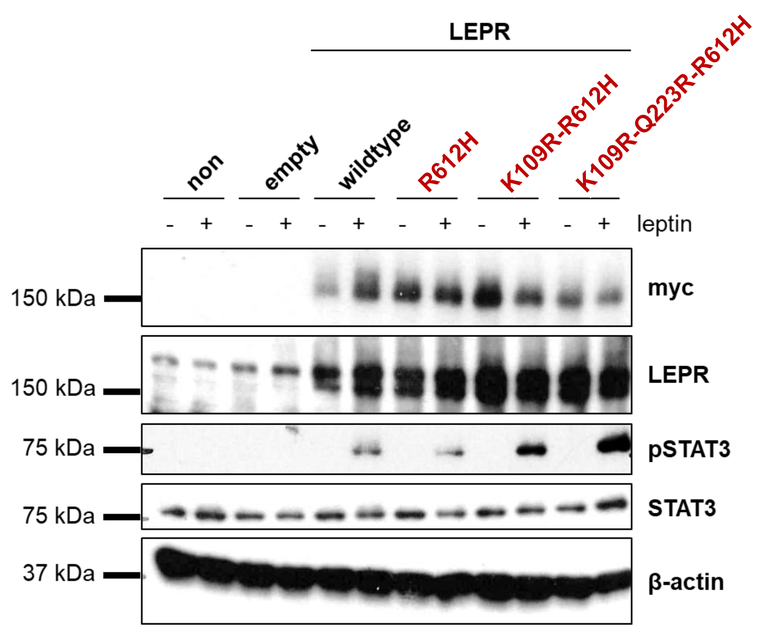


FACS analysis after surface leptin receptor antibody staining. *LEPR*-constructs were C-terminally fused to YFP. All variants, especially the W664R mutant impaired leptin receptor cell surface expression.

4. No additional effect of polymorphisms

All variants found in patient 2 (R612H and the two polymorphisms K109R and Q223R) were mutated in the same *LEPR* vector.

No reduced STAT3 phosphorylation could be observed even in this combination.



Conclusion

The *LEPR* variants Y411del and W664R were not able to induce STAT3 signaling. In case of the W664R variant, this is presumably due to absent membrane translocation. Hence, these variants are a likely cause for the early onset obesity of patient 1. The heterozygous R612H variant, however, appears unlikely to explain the phenotype of patient 2 from our experimental analyses.

References

[1] Allison M et al., Journal of Endocrinology. 2014; [2] Dubern B et al., Biochimie. 2012; [3] Kimber W et al., Endocrinology. 2008. [4] Fong et al., Mol Pharm. 1998.

Supported by the Integrated Research and Treatment Center (IFB) Adiposity Diseases (grant number AD2-7102) and the German Research Foundation (CRC 1052 Obesity Mechanisms C05)

Contact: antje.koerner@medizin.uni-leipzig.de

Disclosure: The authors declare no potential conflict of interest.