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

Franziska Voigtmann1,3, Robert Stein1, Philipp Wolf2, Kathrin Landgraf1,3, Rami Abou Jamra4, Wieland Kiess1, Antje Körner1,3

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

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

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

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