Screening for genetic and structural variation in the *NPY2R* gene in obese children and adolescents.

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1. Introduction

- Neuropeptide Y2 Receptor (NPY2R)
  - 7TM G protein-coupled presynaptic inhibitory receptor
  - Highly expressed in orexigenic NPY/AgRP neurons within the arcuate nucleus
  - Role in energy homeostasis → Inhibitor of NPY-release
    → Indirect regulator of melanocortin signaling
- Interesting candidate gene for obesity
  - *NPY2R* rs1047214, rs2880415 and rs6857715 are associated with severe obesity
  - *NPY2R* null mice are hyperphagic
- Hypothesis
  - Genetic and structural variation in *NPY2R* might influence food intake and weight regulation

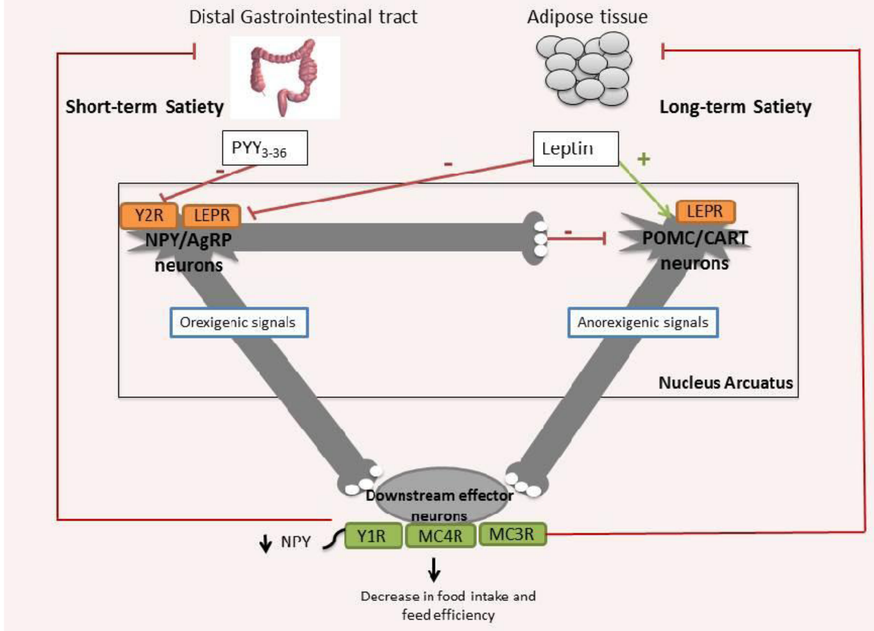

Figure 1: Schematic representation of the Y2 receptor signaling pathway, involved in the regulation of energy balance and food intake.

PYY: Peptide YY; Y2R: Y2 receptor; LEPR: Leptin Receptor; NPY: Neuropeptide Y; AgRP: Agouti-related peptide; POMC: Pro-opiomelanocortin; CART: Cocaine- and amphetamine-related transcript

2. Materials and methods

- Population

Table 1: Population characteristics

	Obese subjects		Lean adults
	Children	Adolescents	
N	171	135	300
Male/female	109/62	39/96	124/176
Age (years)	9,5 ± 0,4	13,6 ± 0,2	34,4 ± 0,3
Weight (kg)	52,4 ± 1,7	95.7 ± 2,5	65,8 ± 0,5
Height (m)	1,37 ± 0,02	1,65 ± 0,01	1,73 ± 0,01
BMI (kg/m²)	28,3 ± 0,4	33,5 ± 0,5	21,9 ± 0,1
BMI Z-score	2,58 ± 0,04	2,6 ± 0,04	N.A.

Mean value ± standard error of mean is shown for all parameters, except N and gender distribution (absolute numbers). N.A., not applicable

- Mutation analysis
  - High-Resolution Melting Curve Analysis on a Lightcycler 480 Real-Time PCR system (Roche)
  - Sanger Sequencing
- *In silico* analysis
  - PolyPhen-2
  - SIFT
  - Conseq 1.1
  - MutPred

  To predict the impact of non-synonymous variants
- Multiplex Amplicon Quantification (MAQ)
  - Detection and analysis of copy number variation (CNV) in the *NPY2R* region

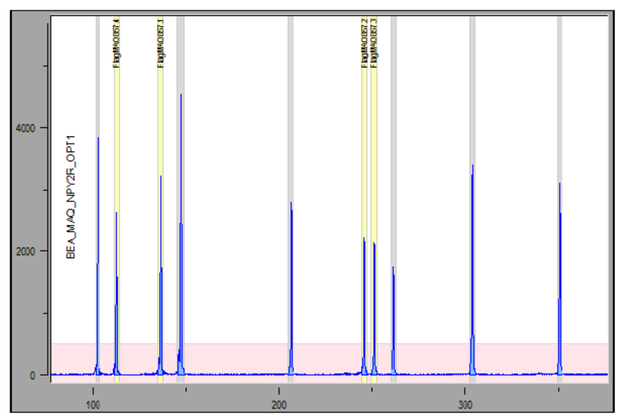

Figure 2: Fragment analysis with MAQ-software generates an electropherogram .

3. Results

- Mutation analysis (Table 2)
  - 2 private synonymous variants in lean adults
  - 3 private synonymous variants in obese cases
  - 1 rare non-synonymous heterozygous variant (F87I) in an obese patient
    → The most interesting variant for further research
- *In silico* analysis of F87I
  - Probably pathogenic impact of F87I on the energy homeostasis
  - Present in the transmembrane segment II in the 7TM receptor, known for its function in ligand-dependent and ligand-independent activation
- MAQ analysis
  - No structural variation in the *NPY2R* region

Table 2: Identified variants in obese cases and lean individuals

Variation			
Gene	Protein	Patients	Controls
c.159c>t (rs234674)	L53L	3	
c.259t>a (rs144160377)	**F87I**	**1**	
c.315g>t (rs158709959)	P105P		1
c.834g>a	A278A		1
c.978c>t (rs138080356)	G326G	1	
c.1110c>a (rs138652181)	G370G	1	

Position of variants on gene and protein levels is shown, as well as their frequency in the obese and lean groups (in absolute numbers). Numbering on gene level is based on cDNA sequence, following the recommendations by the Human Genome Variation Society.

4. Discussion

- Mutation analysis resulted in the identification of 1 rare non-synonymous heterozygous variant F87I in an obese patient. By performing *in silico* analysis, we determined that the F87I variant is probably damaging to the protein structure and might have a disease causing effect. Further functional testing will be necessary to fully understand the impact on NPY2R.
- As we could not identify any CNV in the *NPY2R* region, structural variation in the NPY2R is not likely to cause obesity.

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Diabetes (to include obesity, pathophysiology & epidemiology)

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