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

2. Materials and methods

- Population
  
<table>
<thead>
<tr>
<th>Obese subjects</th>
<th>Lean adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children</td>
</tr>
<tr>
<td></td>
<td>171</td>
</tr>
<tr>
<td>Male/female</td>
<td>109/62</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.5 ± 0.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>52.4 ± 1.7</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.37 ± 0.02</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.3 ± 0.4</td>
</tr>
<tr>
<td>BMI Z-score</td>
<td>2.58 ± 0.04</td>
</tr>
</tbody>
</table>

  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

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

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.