Genetic variation at three genetic loci involved in anorexia nervosa are associated with body weight regulation

Anke Hinney

Department of Psychiatrie, Psychosomatik und Psychotherapie des Kindes- und Jugendalters
Do genetic risk factors for obesity and anorexia nervosa overlap?

- Genetic mechanisms have been described for both obesity and anorexia nervosa.
- The maintenance of a normal body weight is disrupted in patients with anorexia nervosa (AN) for prolonged periods of time.
- Prior to the onset of AN body weight covers the whole BMI range; however, after recovery the BMI distribution is shifted towards the left with lower than average rates of overweight and obesity.
- As such, gene loci involved in body weight regulation may also have an impact on AN and vice versa.
Background

Heritability estimates

Twin, adoption- and family studies revealed substantial heritability for

• Variance of BMI

• Mental disorders, incl. AN


‘Identical Twins Reared Apart’, von Susan L. Farber

Background

Genome wide association studies (GWAS)
Hypothesis: specific variants underlying genetic effects are relevant for 5 mental disorders (Psychiatric Genomics Consortium): autism spectrum disorders, ADHD, bipolar disorder, depression (MDD), and schizophrenia

33,332 cases (5 disorders) and 27,888 controls of European descent

SNPs at 4 loci genome-wide significant (p<5×10^{-8}) in the primary analysis: regions on chromosomes 3p21 and 10q24, and SNPs within the 2 genes of L-type voltage-gated calcium channel subunits (CACNA1C and CACNB2)
GIANT: BMI-Study

- Meta-analysis (GWAS and Metabochip) for BMI
- up to 339,224 individuals
- identification of 97 BMI loci (56 new)
- 2.7% of the variance of BMI explained, genome-wide estimators showed that frequent alleles explain up to 20% of BMI variation
- Role of the central nervous system for obesity susceptibility
- Implications of new genes and metabolic pathways, incl. those relevant for synaptic function, glutamat signaling, insulin secretion/action, energy metabolism, lipid biology and obesity

Locke et al. 2015, Nature. 2015 Feb 12;518:197-206
GIANT: BMI-Study

Genes with potential relevance for mental disorders:

**BDNF** (ADHD)

**GPRC5B** (ADHD, Alzheimer)

**APOE** (Alzheimer)

**PARK2** (Parkinson, ADHD)

Locke et al. 2015, Nature. 2015 Feb 12;518:197-206
Of the 97 BMI loci 35 (binomial P = 0.0019) are in high LD (r2 > 0.7) with one or more GWAS SNPs of the National Human Genome Research Institute (NHGRI) GWAS catalog (P < 5 x 10^{-08})

These SNPs were not only associated with cardio-metabolic phenotypes, but also with

- Alzheimer's disease
- Schizophrenia
- Smoking
- Inflammatory bowel disease
International GWAS Consortia

Wellcome Trust Case Control Consortium (WTCCC)
Tuberculosis, Coronary Heart Disease, Diabetes Mellitus Type 1 and 2, Rheumatoid Arthritis, Morbus Crohn and Colitis Ulcerosa, Hypertension

Bipolar Disorder, Anorexia Nervosa

Genetic Association Information Network (GAIN)
Psoriasis, Diabetic Nephropathy in Diabetes Mellitus type 1

ADHD, Schizophrenia, Bipolar Disorder, Depression

GIANT (Genetic Investigation of Anthropometric Traits)
Body weight, Body height, WHR
A genome-wide association study on common SNPs and rare CNVs in anorexia nervosa

GWAS with 1033 patients with AN, 3733 pediatric controls of European descent

No genome wide significant result

Known candidate genes re-identified: OPRD1 (rs533123, P = 0.0015), SNPs near HTR1D (rs7532266, P = 0.04)

No hint for association of defined CNVs with AN, some rare CNVs only indentified in AN
Background

Largest GWAS for anorexia nervosa

A genome-wide association study of anorexia nervosa

Largest GWAS for anorexia nervosa

International multicenter study funded by the ‘Welcome Trust Case Control Consortium‘ (WTCCC3)

- Coordination: C. Bulik (Chapel Hill/USA) and D. Collier (London/UK)
- N = 2,907 patients with AN (475 of these from Germany) and 14,860 controls (Illumina 660W-Quad)
- 72 SNPs for replication
- Global meta-analysis (discovery and replication): 5,551 AN and 21,080 controls: no genome wide significant result
- 76% of the effects directionally consistent for discovery and replication (P = 4x10^6)

Largest GWAS for anorexia nervosa

Table 2. Global meta-analysis results of SNPs with the greatest evidence of association for the main anorexia nervosa (AN) case-control analysis

<table>
<thead>
<tr>
<th>CHR</th>
<th>POS</th>
<th>MARKER</th>
<th>NEAREST GENE</th>
<th>EA</th>
<th>NEA</th>
<th>EAF</th>
<th>OR</th>
<th>OR_95L</th>
<th>OR_95U</th>
<th>P</th>
<th>I²</th>
<th>N_st</th>
<th>N_sa</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>182794261</td>
<td>rs9839776</td>
<td>SOX2OT</td>
<td>T</td>
<td>C</td>
<td>0.270</td>
<td>1.158</td>
<td>1.095</td>
<td>1.225</td>
<td>3.01E-07</td>
<td>0</td>
<td>27</td>
<td>21857</td>
</tr>
<tr>
<td>4</td>
<td>102267099</td>
<td>rs17030795</td>
<td>PPP3CA</td>
<td>G</td>
<td>A</td>
<td>0.192</td>
<td>1.149</td>
<td>1.082</td>
<td>1.220</td>
<td>5.84E-06</td>
<td>0</td>
<td>24</td>
<td>23111</td>
</tr>
<tr>
<td>8</td>
<td>19584542</td>
<td>rs11204064</td>
<td>CSGALNACT1</td>
<td>G</td>
<td>A</td>
<td>0.477</td>
<td>1.118</td>
<td>1.063</td>
<td>1.176</td>
<td>1.57E-05</td>
<td>0.008</td>
<td>28</td>
<td>21477</td>
</tr>
<tr>
<td>13</td>
<td>23433988</td>
<td>rs1886797</td>
<td>18 kb from SPATA13</td>
<td>C</td>
<td>A</td>
<td>0.218</td>
<td>1.152</td>
<td>1.079</td>
<td>1.229</td>
<td>2.03E-05</td>
<td>0.244</td>
<td>22</td>
<td>18566</td>
</tr>
<tr>
<td>8</td>
<td>19584542</td>
<td>rs9839776</td>
<td>SOX2OT</td>
<td>T</td>
<td>C</td>
<td>0.301</td>
<td>1.133</td>
<td>1.070</td>
<td>1.200</td>
<td>2.18E-05</td>
<td>0.317</td>
<td>25</td>
<td>15827</td>
</tr>
<tr>
<td>13</td>
<td>23433988</td>
<td>rs1886797</td>
<td>18 kb from SPATA13</td>
<td>G</td>
<td>A</td>
<td>0.074</td>
<td>1.193</td>
<td>1.097</td>
<td>1.297</td>
<td>3.96E-05</td>
<td>0</td>
<td>28</td>
<td>21614</td>
</tr>
<tr>
<td>7</td>
<td>106473684</td>
<td>rs2395833</td>
<td>PRKAR2B</td>
<td>T</td>
<td>G</td>
<td>0.334</td>
<td>1.101</td>
<td>1.051</td>
<td>1.154</td>
<td>5.62E-05</td>
<td>0.132</td>
<td>29</td>
<td>26511</td>
</tr>
<tr>
<td>8</td>
<td>80768625</td>
<td>rs1370339</td>
<td>PRKAR2B</td>
<td>C</td>
<td>T</td>
<td>0.472</td>
<td>1.098</td>
<td>1.049</td>
<td>1.149</td>
<td>5.68E-05</td>
<td>0</td>
<td>29</td>
<td>26508</td>
</tr>
<tr>
<td>13</td>
<td>63470128</td>
<td>rs9539891</td>
<td>255 kb from OR7E156P</td>
<td>C</td>
<td>T</td>
<td>0.332</td>
<td>0.891</td>
<td>0.842</td>
<td>0.942</td>
<td>5.88E-05</td>
<td>0</td>
<td>23</td>
<td>20389</td>
</tr>
<tr>
<td>2</td>
<td>225017222</td>
<td>rs1523921</td>
<td>26 kb from CUL3 / 42 kb from FAM124B</td>
<td>T</td>
<td>C</td>
<td>0.210</td>
<td>1.131</td>
<td>1.065</td>
<td>1.201</td>
<td>5.95E-05</td>
<td>0.162</td>
<td>26</td>
<td>21858</td>
</tr>
<tr>
<td>19</td>
<td>11650015</td>
<td>rs206863</td>
<td>ZNF833P</td>
<td>A</td>
<td>G</td>
<td>0.899</td>
<td>0.864</td>
<td>0.804</td>
<td>0.928</td>
<td>6.47E-05</td>
<td>0.076</td>
<td>28</td>
<td>26402</td>
</tr>
<tr>
<td>23</td>
<td>107578961</td>
<td>rs5929098</td>
<td>COL4A5</td>
<td>T</td>
<td>C</td>
<td>0.771</td>
<td>1.135</td>
<td>1.066</td>
<td>1.210</td>
<td>8.37E-05</td>
<td>0.002</td>
<td>29</td>
<td>19249</td>
</tr>
<tr>
<td>7</td>
<td>146565029</td>
<td>rs6943628</td>
<td>CNTNAP2</td>
<td>A</td>
<td>G</td>
<td>0.097</td>
<td>1.161</td>
<td>1.077</td>
<td>1.251</td>
<td>9.38E-05</td>
<td>0</td>
<td>29</td>
<td>26377</td>
</tr>
</tbody>
</table>

Abbreviations: CHR, chromosome; POS, position in hg18; EA, effect allele; NEA, non-effect allele; EAF, effect allele frequency; OR, odds ratio; OR_95L, lower 95% confidence interval; OR_95U, upper 95% confidence interval; P, P-value; I², measure of heterogeneity; N_st, number of contributing studies; N_sa, number of contributing samples.

All genes expressed in mouse brain

Largest GWAS for anorexia nervosa - obesity loci -

Comparison of 76 (53 independent) SNPs of the AN GWAS with 89 established BMI/obesity SNPs revealed $P < 0.05$ for 5 SNPs (in *NEGR1*, *PTBP2*, *TMEM18*, *FTO* and *MC4R*)

26 of the 53 SNPs had the same direction of effect in AN and BMI/obesity (binomial $P = 1$)

13 (9 independent) of 15 SNPs associated with extreme obesity available; 4 of the 9 had the same direction of effect for AN and obesity (binomial $P = 1$)
Cross-trait analysis

• Cross-trait analysis of the 1000 SNPs with the lowest p-values from a GWAS for AN (GCAN, Boraska et al., 2014) for evidence of association in the largest published GWAS meta-analysis for BMI variation (GIANT; Locke et al., 2015)

• We detected significant association (p-values < 5x10^{-05}, Bonferroni corrected p < 0.05) for 9 SNPs at 3 independent chromosomal loci (chromosomes 2, 10 and 19)
### Results

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>SNP 1</th>
<th>Intron</th>
<th>Rank in AN GWAS</th>
<th>Rank AN GWAS</th>
<th>AN effect allele / frequency</th>
<th>OR (SE)</th>
<th>p-value for AN risk</th>
<th>Frequency of reference allele in AN cases</th>
<th>AN risk (odds ratio) for reference allele</th>
<th>Bonferroni corrected p-value</th>
<th>Direction of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>SNP 1</td>
<td>Intron</td>
<td>201</td>
<td>A / 0.33</td>
<td>1.14 (0.04)</td>
<td>7.74 x 10^{-9}</td>
<td>A / 0.34</td>
<td>0.0157</td>
<td>2.47 x 10^{-6} / 3.45 x 10^{-9} / 0.043</td>
<td>0.0025</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>SNP 2</td>
<td>Intron</td>
<td>190</td>
<td>G / 0.74</td>
<td>0.87 (0.03)</td>
<td>7.28 x 10^{-9}</td>
<td>A / 0.25</td>
<td>0.0162</td>
<td>4.25 x 10^{-6} / 5.8 x 10^{-9} / 0.022</td>
<td>0.0043</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>SNP 3</td>
<td>Intron</td>
<td>177</td>
<td>C / 0.75</td>
<td>0.87 (0.04)</td>
<td>6.79 x 10^{-9}</td>
<td>C / 0.75</td>
<td>-0.0171</td>
<td>4.58 x 10^{-9} / 1.03 x 10^{-9} / 0.009</td>
<td>0.0046</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>SNP 4</td>
<td>Distant 5'</td>
<td>409</td>
<td>T / 0.70</td>
<td>0.88 (0.03)</td>
<td>0.0002</td>
<td>T / 0.67</td>
<td>-0.015</td>
<td>5.41 x 10^{-6} / 6.4 x 10^{-9} / 1.24 x 10^{-9}</td>
<td>0.0054</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>SNP 5</td>
<td>Intron</td>
<td>709</td>
<td>A / 0.48</td>
<td>0.90 (0.03)</td>
<td>0.0003</td>
<td>A / 0.49</td>
<td>-0.0134</td>
<td>1.08 x 10^{-6} / 1.8 x 10^{-9} / 2.27 x 10^{-9}</td>
<td>0.0108</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>SNP 6</td>
<td>Intron</td>
<td>444</td>
<td>A / 0.46</td>
<td>0.89 (0.03)</td>
<td>0.0002</td>
<td>A / 0.46</td>
<td>-0.0131</td>
<td>2.48 x 10^{-9} / 9.54 x 10^{-9} / 9.39 x 10^{-9}</td>
<td>0.0248</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>SNP 7</td>
<td>Distant 5'</td>
<td>412</td>
<td>A / 0.46</td>
<td>0.89 (0.03)</td>
<td>0.0002</td>
<td>A / 0.46</td>
<td>-0.0125</td>
<td>3.57 x 10^{-6} / 1.98 x 10^{-9} / 8.21 x 10^{-9}</td>
<td>0.0357</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>SNP 8</td>
<td>Distant 5'</td>
<td>248</td>
<td>T / 0.70</td>
<td>0.88 (0.03)</td>
<td>9.45 x 10^{-9}</td>
<td>T / 0.67</td>
<td>-0.0159</td>
<td>3.76 x 10^{-6} / 0.006 / 2.46 x 10^{-9}</td>
<td>0.0376</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>SNP 9</td>
<td>Intron</td>
<td>401</td>
<td>G / 0.46</td>
<td>0.89 (0.03)</td>
<td>0.0002</td>
<td>A / 0.54</td>
<td>0.0124</td>
<td>4.61 x 10^{-9} / 2.80 x 10^{-9} / 0.008</td>
<td>0.04612</td>
<td>+</td>
</tr>
</tbody>
</table>

*Note: All p-values are corrected for multiple testing.*

**Genetic variation at three genetic loci involved in anorexia nervosa are associated with body weight regulation.**
Results

- Interestingly, all risk alleles were directionally consistent for AN and higher BMI/obesity.
- None of the genes nearest to these SNPs had previously been associated with AN or obesity.
- Information on the function of 3 of these 4 genes is sparse.
- Two genes might be biologically plausible as they are involved in BDNF signaling pathways.
- Sex specific analyses revealed that the most significant result for BMI originated predominantly from females
  - SNP 1; \( p_{\text{females}}: 3.45 \times 10^{-07} / p_{\text{males}}: 0.043 \)
- The look-up of the 56 BMI loci in the AN GWAMA did not reveal significant findings.
Summary

• In a cross-trait analysis three chromosomal loci with potential relevance for both AN and obesity were detected.
• Their role in both traits was substantiated by our sex specific analyses; and the finding that two genes are potentially involved in BDNF regulation.
• Further in depth molecular genetic and biological analyses are essential to understand the relevance of these loci and the genes they contain in the etiology of AN and in obesity.
Thank you!

Essen: Anna-Lena Volckmar, Jochen Antel, Johannes Hebebrand
Jena: André Scherag, Miriam Kesselmeier
Regensburg: Iris M. Heid, Thomas W. Winkler
Aachen: Beate Herpertz-Dahlmann
Hannover: Martina de Zwaan
Heidelberg: Wolfgang Herzog
Dresden: Stefan Ehrlich
Tübingen: Stephan Zipfel
Würzburg: Karin Maria Egberts
Utrecht, The Netherlands: Roger Adan, Marek Brandys
Cambridge, UK: Eleftheria Zeggini
Chapel Hill, NC, USA: Cynthia Bulik
London, UK: David Collier

Consortia: GCAN, WTCCC, GIANT