

Network: Obesity and Related Disorders**Project: Identification of Candidate Genes for Human Obesity**

Johannes Hebebrand - University Duisburg-Essen - johannes.hebebrand@uni-duisburg-essen.de

Introduction

The aim is to identify candidate genes for obesity (leanness) in humans. We will analyse human functional and positional candidate genes in an exploratory fashion to identify those genes that are “promising” and thus require confirmation within other work blocks of our Network. Our candidate genes will be derived from several sources including animal models, expression studies, and findings in co-morbid disorders. In addition, in collaborations with the Max Delbrück Centre for Molecular Medicine (MDC, Berlin) and associated industrial partners including *deCODE genetics* (Reykjavik, Iceland) and *IntegraGen* (Evry, France) results obtained in genome wide scans will be followed up.

Microsatellite analyses, mutation screens of candidate genes, association and transmission disequilibrium tests have been performed for many genes (see references). We were the first group to describe the spectrum of obesity-relevant mutations in the melanocortin-4 receptor gene (*MC4R*). This gene is as yet considered the most important obesity gene, as functionally relevant mutations occur in 2-4 % of extremely obese individuals [e.g. 1, 2]. Our initial finding led to the analysis of *MC4R* in more than 10 subsequent collaborative studies [e.g. 3-12]. Extensive networking with partners with expertise in different research areas was established for these analyses. We were the first group that quantified the effect of *MC4R* mutations in obesity [10]. According to our family studies male and female carriers of functionally relevant mutations have a body mass index (BMI) that is approx. 1.3 and 2.5 standard deviations higher than that of their wild-type relatives, corresponding to 4 and 9.5 kg/m² in middle aged adults, respectively. We view our *MC4R* studies as prototypical for future studies of confirmed candidate genes.

In the course of NGFN-1 we established a fruitful cooperation with Professor Meitinger at the National Research Center for Environment and Health (GSF, Munich) which has resulted in joint publications. We have passed on 3,448 DNA samples to Munich; these were transferred onto 384-well plates and collaborative high-throughput single nucleotide polymorphism (SNP) typing was established. In collaboration with Professor Nürnberg and Dr. Saar (MDC, Berlin) genome scans for three different phenotypes (childhood obesity, attention deficit/hyperactivity disorder and constitutional delay of growth and puberty) were performed. To this end, high-quality DNA was provided according to SOPs. Over the past nine years biometrical analyses have been successfully performed in collaboration with the platform for Genetic Epidemiological Methodology (GEM) in Marburg (Professor H. Schäfer). In a very efficient cooperation with Drs. Platzer/Reichwald (Institute of Molecular Biotechnology, Jena) sequencing of several functional candidate genes and respective 5'- and 3'-flanking regions was performed. Together with Professor Brockmann (Humboldt-University, Berlin) we analysed five murine positional candidate genes underlying quantitative trait loci (QTLs) for increased body weight and obesity that Professor Brockmann and co-workers identified by differential gene expression analyses in adipose tissues. We hypothesize that the orthologous human genes will also show genetic variation affecting body weight. At *deCODE genetics* new human genes relevant for obesity in the Icelandic population have been identified by a genome scan. These genes will be re-analysed in our German samples in order to assess their impact and/or validate the original findings in a second European population. A formal agreement has been signed between both groups and in

addition, *deCODE* was provided with DNA of 2,967 German individuals.

Results/Project Status**Candidate gene studies (mutation screen, genotyping)**

- a) *Melanocortin 4 receptor gene (MC4R)*:
 - 1) Genotyping of 7,937 individuals from the population-based KORA sample confirmed the negative association of the I103-allele of SNP rs2229616 with obesity [9, 11].
 - 2) CE-SSCP screen of the *MC4R* coding sequence (CDS) in 4,261 individuals of the KORA-S2000 cohort, functional characterization of the detected mutations, evaluation of the relevance of the mutations for body weight regulation which in this sample are representative of the general population (*Hinney et al., in preparation*).
 - 3) Genotyping of *MC4R* sequence variations in approx. 1,200 individuals with cardiovascular disease. Analysis of association of these variations with serum lipid levels and blood pressure (*Broenner et al., resubmitted*).
- b) Re-sequencing of *Mc4r* and flanking regions in mouse lines prone/resistant to diet-induced obesity, identification of 111 sequence variations including 6 variations in the CDS, functional analyses in collaboration with Dr. Biebermann (Charité, Berlin) (*Reichwald et al., in preparation*).
- c) Re-sequencing of putative promoter regions and/or downstream flanking regions of five murine positional candidate genes in selected mouse lines totaling 480kb in collaboration with Professor Brockmann (Humboldt-University, Berlin).
- d) Mutation screen of the melanin-concentrating hormone receptor 1 gene (*MCHR1*) and association analysis of two coding SNPs. Identification of 11 infrequent variations, 2 SNPs in the CDS and 18 SNPs in gene flanking regions. Association and transmission disequilibrium with obesity was found for several SNPs in German obese children and adolescents but could not be confirmed in two additional epidemiological German samples and in Danish, French and American study groups [13].
- e) Screen for mutations in the CDS of the gene encoding diacylglycerol O-acyltransferase 2 (*DGAT2*), identification of 10 novel and 3 known mutations, association tests for all mutations and PDT in 165 families comprising at least two obese children and both parents were negative (*Friedel et al., in preparation*).
- f) *Galanin and galanin-1 receptor genes (GAL, GAL1R)*: screen for mutations in the CDS, identification of novel mutations; association studies and TDTs were negative [14].
- f) *Uncoupling protein 3 gene (UCP3)*:
 - 1) Analysis of SNP rs1800849 in *UCP3* by high-throughput genotyping (MALDI-TOF, together with Professor Meitinger, GSF) in 184 obese cases and 184 underweight controls resulted in a positive association (p=0.0003). Confirmation was achieved by genotyping of 368 obesity trios; (156/123 transmissions/non-transmissions) p = 0.048; and unexpectedly not confirmed by genotyping of obesity-quartets (PDT) p: 0.63. Genotyping of rs1800849 and two additional SNPs (rs1800006, rs2075577) in the epidemiological population-based KORA K46/04 cohort (Drs. Wichmann/Illig, GSF, Munich) was performed. For the analyses in the KORA sample, a linear model was used for BMI and logBMI, respectively. A test for the

categorical predictor variable 'genotype' was negative. Combined analysis by co-evaluation of sex and ages was negative; no differences between the different genotypes, no phenotype-genotype interaction were detected. All exploratory comparisons in the KORA sample were also negative. Hence, an effect of the rs1800849 on body weight regulation might only be relevant for children and adolescents. The initial finding might also just have been spurious.

- 2) In parallel, functional *in vitro* studies with promoter constructs are performed to evaluate the relevance of the promoter SNP (rs1800849; Dr. Klingenspor, Philipps-University, Marburg.)

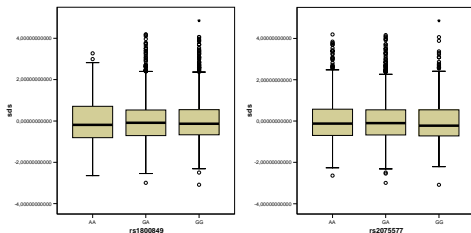


Fig 1 and 2: Boxplots of BMI-SDS values for UCP3 genotypes based on SNPs rs1800849 and rs2075577 (corrected for age and gender).

SNP-based genome wide linkage scan

In a genome wide linkage scan using a high density marker panel (10K Affymetrix SNP Chip) performed in approx. 300 obesity families, results obtained in our previous, microsatellite (MS) based genome wide linkage scan [15] were confirmed. For example, linkage regions identified on chromosomes (chr) 8 and 11 in the MS scan were validated, however, the higher information content (0.97 vs 0.77 in the MS based scan) of the 10K SNP array results in higher linkage signals (Figure 3).

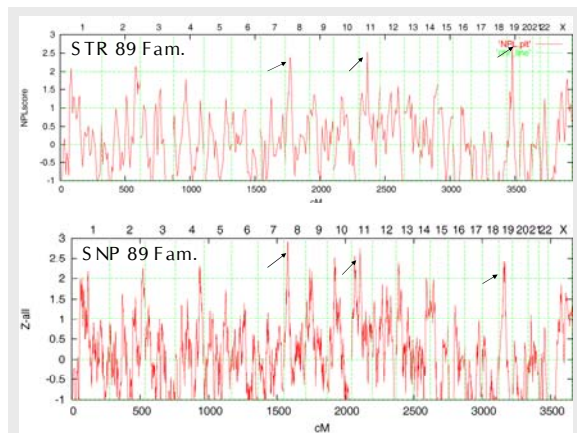


Fig 3: Comparison between 10K-Affymetrix SNP Chip and a conventional microsatellite linkage scan of 89 obesity families. The top panel depicts Merlin NPL scores obtained in the conventional scan, below results of the 10K SNP array are shown. Arrows mark linkage peaks on chromosomes 8, 11 and 19. STR: short tandem repeats – microsatellites

Outlook

Candidate gene analyses of selected SNPs/alleles in the genes *GAL*, *GALR1* and *DGAT2* did not show association with body weight. For the *MCHR1* gene, association of several SNPs with obesity in German children and adolescents could not be confirmed in study groups from Denmark, France and Northern America.

Furthermore, our results pertaining to *MC4R* do not indicate that adults of a population-based sample who are heterozygous for *MC4R* mutations with *impaired function*

have increased BMI-SDS compared to carriers of *wild type like* variations or homozygotes for the wild type allele. Interestingly, we found a lower prevalence of *MC4R* mutations with *impaired function* in study groups of obese adults compared to our and other previous screens in extremely obese children and adolescents. This might imply an age dependent prevalence effect. Otherwise it might indicate that we will only be able to detect a relative accumulation of such mutations in groups of even more extreme phenotypes like an age- and gender-specific BMI percentile of 99 or above.

Nevertheless we were able to demonstrate that the presence of such mutations does not automatically or with high probability lead to an elevated BMI, as might have been concluded from previous studies of (extremely) obese study groups. According to the given data, additional genetic and environmental factors might be considered relevant for the development of obesity in heterozygotes for *MC4R* mutations. Currently we are following up on results of a genome wide 10K Affymetrix SNP Chip scan for obesity (see above). Five chromosomal regions show suggestive linkage. Fine-mapping of two regions on chr 4 and 17 comprising 7 and 9 Megabase pairs, respectively, which contain more than 200 referenced positional candidate genes is being prepared; identified genes might exert major effects. In addition, we will participate in a genome wide association scan based on the 500K Affymetrix SNP Chip to be performed in KORA samples.

Lit.: 1. Hinney A et al. Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. *J Clin Endocrinol Metab.* 1999 Apr;84(4):1483-6; 2. Hinney A et al. Melanocortin-4 receptor gene: case-control study and transmission disequilibrium test confirm that functionally relevant mutations are compatible with a major gene effect for extreme obesity. *J Clin Endocrinol Metab.* 2003 Sep;88(9):4258-67; 3. Sina M et al. Phenotypes in three pedigrees with autosomal dominant obesity caused by haploinsufficiency mutations in the melanocortin-4 receptor gene. *Am J Hum Genet.* 1999 Dec;65(6):1501-7; 4. Hebebrand J et al. Genetic predisposition to obesity in bulimia nervosa: a mutation screen of the melanocortin-4 receptor gene. *Mol Psychiatry.* 2002;7(6):647-51; 5. Mart A et al., A novel nonsense mutation in the melanocortin-4 receptor associated with obesity in a Spanish population. *Int J Obes Relat Metab Disord.* 2003 Mar;27(3):385-8; 6. Herpertz S et al. Binge eating as a phenotype of melanocortin 4 receptor gene mutations. *N Engl J Med.* 2003 Aug 7;349(6):606-9; 7. Weide K et al. Hyperphagia, not hypometabolism, causes early onset obesity in melanocortin-4 receptor knockout mice. *Physiol Genomics.* 2003 Mar 18;13(1):47-56. 8. Hebebrand J et al. Binge-eating episodes are not characteristic of carriers of melanocortin-4 receptor gene mutations. *Mol Psychiatry.* 2004;9:796-800; 9. Geller F et al. Melanocortin-4 receptor gene variant I103 is negatively associated with obesity. *Am J Hum Genet.* 2004;74:572-81; 10. Dempfle A et al. Large quantitative effect of melanocortin-4 receptor gene mutations on body mass index. *J Med Genet.* 2004;41:795-800; 11. Heid IM et al. Association of the 103I *MC4R* allele with decreased body mass in 7937 participants of two population based surveys. *J Med Genet.* 2005;42:e21; 12. Zakel UA et al. Prevalence of melanocortin 4 receptor (*MC4R*) mutations and polymorphisms in consecutively ascertained obese children and adolescents from a pediatric health care utilization population *Klin Padiatr.* 2005;217:244-9; 13. Wermter AK et al. Mutation analysis of the *MCHR1* gene in human obesity. *Eur J Endocrinol.* 2005;152:851-62; 14. Schäuble N et al. Human galanin (*GAL*) and galanin 1 receptor (*GALR1*) variations are not involved in fat intake and early onset obesity. *J Nutr.* 2005;135:1387-92; 15. Saar K et al. Genome scan for childhood and adolescent obesity in German Families *Pediatrics.* 2003; 111:321-7.