

**Network: Obesity and Related Disorders****Project: Identification of Candidate Genes for Human Obesity in a Mouse Model of Morbid, Polygenic Obesity**

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**Introduction**

In most industrialized countries, the prevalence of obesity and its life-shortening complications (hypertension, cardiovascular complications, type-2 diabetes) have increased markedly during the last 20 years. Thus, obesity has become one of the most important contemporary health problems. The aim of this project is to identify candidate genes that are responsible for the polygenic obesity in mouse strains and to define their interaction. Also, it is intended to define the interaction of the gene variants with exogenous factors, e.g. the dietary fat. For further verification of the candidate genes in obesity, their role in metabolism, energy balance, and signal transduction will be characterized.

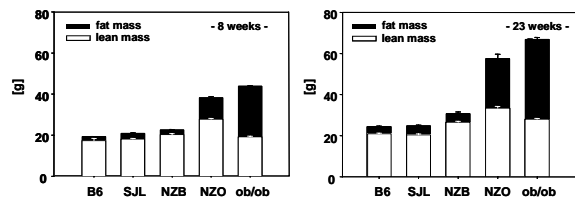
The available evidence indicates that a considerable portion of the genes responsible for rodent obesity also plays a role in the human disease, as exemplified in the case of leptin/leptin receptors, melanocortin receptor-4 (MC4R), and peroxisome proliferator activated receptor-gamma (PPAR-gamma). However, because the majority of the genes contributing to common human obesity is still unknown, studies of natural obesity alleles in mice by QTL mapping and other resources permit efficient identification of the full range of genes and alleles that cause human obesity, including the analysis of gene-gene and gene-environment interactions. Furthermore, the employment of inbred mouse strains eliminates the intra-individual heterogeneity in the genetic background that heavily contributes to the phenotypic variance of human obesity. This project will therefore facilitate and accelerate the search for human obesity genes.

The mouse model used in this project is the *New-Zealand obese* (NZO) mouse, which closely resembles the human polygenic metabolic syndrome (1), and which can also be used to analyze gene-gene and gene-environment interactions. For the characterization of the obesity and the metabolic syndrome of NZO we have generated two different backcross population of NZO with the lean SJL (*Swiss/Jim Lambert*) strain. With these backcross populations, we have defined several QTL for obesity, diabetes (decompensated hyperglycemia and hypoinsulinemia), and hypercholesterolemia (2-5). We have shown that some of these QTL interact with the diet in that their penetrance depends on the dietary fat content (4). Furthermore, the data have indicated that at least 20 obesity-QTL are present in NZO mice, and that some of these may be present in other, less obese mouse strains. With whole-genome DNA arrays (global screening), we identified approximately 60 genes whose expression is modified in tissues from obese or diabetic mice (6).

For the identification of variant genes (candidate genes) located in the different QTL of the NZO strain several parallel approaches are currently employed. Outcrosses of NZO with different mouse strains, generation of haplotype maps of the corresponding strains, bioinformatics analysis, expression studies and sequencing of candidates within critical regions will finally lead to the identification of gene variants for obesity and the metabolic syndrome.

**Results****Physiological characterization of different mouse strains**

In order to facilitate the identification of candidate genes a detailed physiological characterization of the different parental strains (NZO, NZB, and C57BL/6) was performed. This characterization includes the determination of the body weight development, quantitative assessment of energy balance, and body composition analysis (NMR). As illustrated in Fig. 1 already at the age of eight weeks NZO mice exhibit a higher body weight and body fat content than the other strains and adiposity increased thereafter. For the assessment of energy balance we are currently performing the measurement of food intake, and assimilation, energy expenditure, locomotor activity, body temperature, and metabolic rate by indirect calorimetry of NZO mice in comparison to the control strains C57BL/6 and NZB.



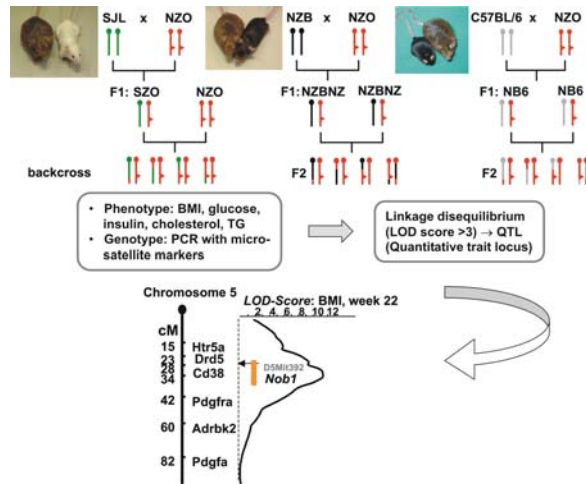
**Fig 1:** Comparison of body weight, lean mass, and fat mass of NZO, NZB, C57BL/6 (B6), and B6.V-*Lep<sup>ob</sup>/J* (ob/ob) mice.

**Outcrosses of the NZO mouse with different other strains**

In order to obtain the required phenotypic information, two outcrosses with the NZO mouse were performed. We generated about 600 F2 mice per cross. As breeding partners we used the NZB (*New Zealand black*) strain, as the next relative of NZO which does not develop obesity, and C57BL/6 as the most widely distributed reference strain. Phenotypic characterization and determination of the genotypes lead to the verification and dissection of known obesity QTL. In addition, by comparing the results obtained with different breeding partners we are able to deduce which partner inserted the disease allele responsible for the adipogenic phenotype.

**Generation of haplotype maps**

In most inbred mouse strains, polymorphic and non-polymorphic segments (haplotypes) are usually large (2-10 Mbp), and, because of the availability of physical maps, can be determined by genotyping of microsatellite markers (QTL regions only, with a total of approximately 800 microsatellite markers) and by SNP analyses. For gene variants that originated before the respective line was generated by inbreeding (e.g. in NZO compared with the NZB strain) haplotype maps of the different strains can be used to exclude large portions of a QTL. Haplotype maps of the different parental strains were generated and together with results obtained from different outcross populations lead to the reduction of critical regions. For instance, the obesity locus *Nob1* on chromosome 5 contains about 300 genes, the haplotype maps allow a reduction of the number of genes to less than 70.



**Fig 2:** Strategy for the localization of susceptibility loci for obesity, diabetes, and hypercholesterolemia in the mouse genome. In a backcross population of the lean SJL and the obese NZO mice, and in outcross populations of the NZO x NZB and NZO x C57BL/6 several chromosomal regions associated with obesity (e.g. *Nob1*), increased blood glucose levels or increased cholesterol levels were identified by analysis of phenotypes (e.g. body weight) and genotypes (determined with microsatellite markers).

**Bioinformatics approach**

The recent advances in sequencing whole genomes provide an opportunity for comparative analysis of human and mouse genes and their respective regulatory networks involved in metabolic control. Therefore, we have assembled physical gene maps of QTL for obesity and related phenotypes from various mice crossbreeding experiments, and from linkage analyses for human obesity (7). Interestingly, the distribution of the obesity QTL within both the mouse and human genome is not homogenous, because multiple QTL cluster around specific areas of certain chromosomes. This strongly suggests the occurrence of obesity hot spots in the genome of both mice and men. In fact, there is a considerable (70%) positional overlap between the  $\approx 150$  mouse obesity QTL and the present  $\approx 180$  human candidate loci with reported linkage to obesity-related phenotypes. These specific areas are currently being investigated by annotation and haplotype mapping, and will serve as a further source for obesity candidate genes. It is likely that a fine mapping of these areas to the single gene level will reveal novel key regulators for body weight control.

**Gene expression analyses**

In order to identify candidate genes based on differential expression we are currently performing gene expression analysis of candidates located within polymorphic regions by

real-time PCR on the RNA of various tissues of NZO, NZB, C57BL/6, and SJL kept on standard versus high-fat diet.

**Sequencing approach**

Obesity QTL-associated candidate genes located in polymorphic regions are investigated by automated DNA sequencing of genomic DNA derived from the obese NZO strain, and from other related and non-related mouse strains (NZB, C57BL/6, SJL). By sequencing the promoter region, intron-exon boundaries, and the coding sequences, respectively, we will gather data that allow both, a functional evaluation of the gene variant, and the assembly of a high resolution haplotype map for the different mouse strains.

**Outlook**

In the ongoing project we will employ the above listed strategies to identify the gene variants within our mouse model that are involved in the development of obesity, in insulin sensitivity, regulation of food intake and thermogenesis. For further verification of the functional role of the gene variants in obesity, their interaction with dietary factors will be characterized. In cooperation with other partners within this network genes identified in the mouse model will be tested for their association with the human disease in cohorts of individuals with the metabolic syndrome.

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