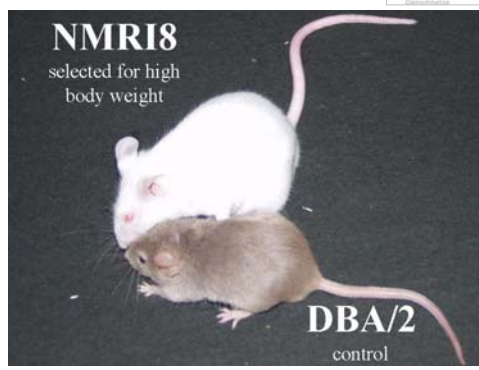
Structural model analysis for adiposity in mice

Gudrun A Brockmann[1], Renhua Li[2], Christina Neuschl[1], Gary A Churchill[2]

[1]Humboldt-Universität zu Berlin, Invalidenstrasse 42, Berlin, Germany, [2]The Jackson Laboratory, Bar Harbor, Maine, USA

Background

Body weight is a complex trait consisting of lean and fat mass, bones, and body fluids. In the cross NMRI8 x DBA/2, QTLs for body weight as a whole as well as for fat pad weights, and muscle weights have been mapped across the genome. Interaction with sex, and epistatic interaction between genetic loci have been identified [1]. Some QTLs affecting body weight, fat, and muscle weights are co-localized in small chromosomal regions which might indicate pleiotropic effects [2]. How the different loci contribute to different traits directly and how the traits influence each other is scarcely analyzed. Here we use the method of structural equation modeling to find the relationship between different genetic loci and different phenotypes influencing body composition in mice.



Results

The final Structural Equation Model (SEM) shows that adiposity is influenced by loci that either affect fat deposition alone or have pleiotropic effects on fat tissue and muscle development (Fig.1).

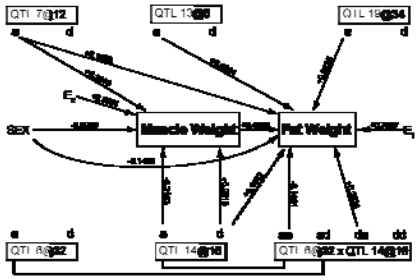


Figure 1. Graphical representation of the final SEM for the direct and indirect actions and interactions of different variables contributing to high adiposity in the NMRI8xDBA/2 intercross population. The QTL positions are given as chromosome number and cM position separated by @. The x indicates epistatic interaction between two genetic loci found by a screen for pair-wise interactions in the F_2 population.
The **arrows** indicate paths between variables. The direction of effects is given by the **edges**; the **values** represent the path coefficients. These are standardized values which represent relative effect sizes. **Positive values** indicate increasing and **negative values** reducing effects of the NMRI8 allele compared to the DBA/2 allele effect in the F2 population. **E1** and **E2** denote unobserved residual effects.

The correlation analysis shows that the relationship between Gonadal Fat Pad Weight (GFPW) and Muscle Weight (MW) is influenced by the Muscle Weight (Fig. 2).

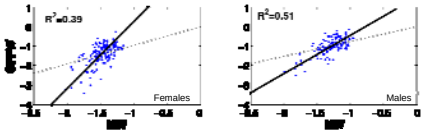


Figure 2. Relationship between Gonadal Fat Pad Weight (**GFPW**) to Muscle Weight (**MW**) for female and male animals. Both phenotypes are log transformed to obtain maximal linear relationships. The **dotted line** indicates the ratio standard of constant adiposity index, the **solid line** is the regression of GFPW on MW.

The QTL analysis identified QTLs for Gonadal Fat Pad Weight and Muscle Weight and significant changes in LOD scores after fitting SEX and Muscle Weight as covariates (Fig. 3).

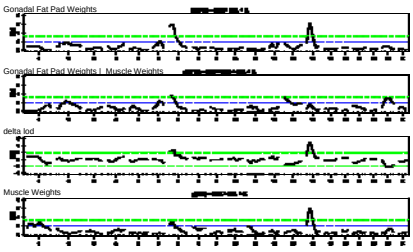


Figure 3. Genome-wide scans for Gonadal Fat Pad Weights and Muscle Weights in the F_2 population. **(A)** Genome-wide scan for Gonadal Fat Pad Weights with sex as an additive covariate, **(B)** genome-wide scan for Gonadal Fat Pad Weights with sex and Muscle Weight as additive covariates, **(C)** difference in LOD scores between scans (A) and (B), showing which QTLs change in the LOD score significantly (delta LOD>2), when Muscle Weight is used as an additional additive covariate, **(D)** genome-wide scan for Muscle Weight with sex as a covariate.

Table 1 on the right side gives the proportion of the phenotypic F_2 variance explained by the coefficients given in Fig 1.

Variable	Predictor	Effect	F_2 variance (%)	t-statistic
Gonadal Fat Pad Weight	Sex		3.4	3.04
	Muscle weight		9.9	8.79
	QTL 7@12	a	4.1	3.64
	QTL 14@16	d	3.8	2.82
	QTL 19@34	a	1.9	1.69
	QTL 13@6	a	2.2	2.03
	6@32 * 14@16	a*a	3.8	2.20
	6@32 * 14@16	d*a	6.0	3.25
Muscle Weight	Sex		2.3	1.71
	QTL 7@12	a	3.7	2.77
	QTL 14@16	a	7.4	3.24
	QTL 14@16	d	6.5	2.85

Initial path models that were tested are

- Muscle weight → Gonadal fat pad weight
- Muscle weight ← Gonadal fat pad weight
- Muscle weight ↔ Gonadal fat pad weight

Only model 1 was significant.

Synopsis

As a polygenic model for obesity, we are using the mouse line NMRI8 which has been long term-selected for high body weight at 8 weeks. Recently, we have mapped QTLs for body weight, adiposity, and muscle weights in an intercross population between the mouse lines NMRI8 and DBA/2. The traits were measured at 6 weeks, when animals became fertile and have finished the fastest growth phase. Using the technique of structural model analysis, we considered body weight as lean mass and fat mass and were able to distinguish genetic loci that affect adiposity from those that affect lean mass. The results show that the NMRI8 alleles of the selection line on chromosomes 7 and 14 have pleiotropic positive effects on both muscle and fat tissue mass, while a locus on chromosome 13 contributed to the selection response only by increased fat deposition. The fat mass was also affected by a complex pattern of interaction between loci on chromosomes 6 and 14. The sex of individuals affected the fat mass either directly or indirectly via the muscle as a mediator. The analysis sheds new light on the action of genes controlling body weight as composite trait of fat and muscle tissues.

Structural Equation Model (SEM)

SEM is a descriptive and inferential tool to investigate the simultaneous effects of QTLs on multiple phenotypes and interactions among these phenotypes. SEM are also known as path models. The model structure is represented as directed graph between measured variables. SEM are an extension of standard multiple regression techniques which emphasizes the correlation structure of continuously distributed variables. Any given variable may be both a response and a predictor.
The development of a Structural Equation Model is performed in five steps:

1. Identification of QTLs for individual phenotypes,
2. Identification of pleiotropic QTLs,
3. Definition of an initial path model,
4. Assessment of the model,
5. Refinement of the model.

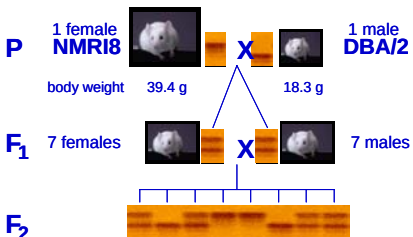
Model refinement and assessment are often carried out iteratively. The final model provides a reasonable description of the data if several standards are fulfilled [3].

Animal model

Population: 275 F_2 mice of the cross NMRI8xDBA/2.

Diet: Animals were fed *ad lib.* a standard maintenance (9% energy from fat) or a high fat diet (45% energy from fat) from 3-10 weeks.

Measurements: Body weight, gonadal fat pad weight (**GFPW**), and muscle weight (quadriceps, **MW**) at 6 weeks.



Data analysis

QTL interval mapping

Multi locus genome scans were performed with RQTL. Pseudomarkers at 2 cM distances were introduced with 128 imputations [4]. Experiment-specific significance thresholds were determined by 1000 permutations (genome-wide significant at p<0.1, genome-wide suggestive at p<0.1).

Structural Equation Modeling

SEM was carried out with SAS using PROC CALIS (SAS, Cary, North Carolina, USA). QTLs were represented by the nearest marker to the LOD peak. Our structural equation modelling included additive (a) and dominance (d) effects of each of the QTLs as well as a*a, a*d, d*a, and d*d epistatic interactions.

References

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