DISSECTION OF THE COMPLEXITY OF POLYGENIC TRAITS BY CHROMOSOME SUBSTITUTION STRAINS IN MICE

M.R. Bevova, U. Renne and G.A. Brockmann

Research Institute for the Biology of Farm Animals, Wilhelm-Stahl-Allee 2,
D-18196 Dummerstorf, Germany

INTRODUCTION

Growth and obesity are polygenic traits which are regulated by many genes with relatively small effects under the influence of the environment. The genes can act individually or in interaction with each other. Traits of this nature are named complex traits and the individual loci affecting the trait are quantitative trait loci (QTL).

The mouse is clearly a key model animal for dissecting the molecular mechanisms underlying biological processes of body weight regulation both, in humans and in animals. Although there are many differences between species, general pathways responsible for body weight regulation are likely conserved in mammalian evolution. During the last years, mice have been used to unravel novel components that contribute to body weight regulation which also act in humans and farm animals. Using the genome synteny between species, data obtained in mice may be used for research in humans and farm animals and visa versa.

Genetic studies based on natural pedigrees in humans and on crossbred populations in mouse, rat, and farm animals have led to the identification of various genetic components responsible for weight control (rev. by Pérusse *et al.,* 2001). In mice, crosses between phenotypically extremely different inbred and selected lines have been successfully used for the mapping of QTLs influencing body weight and composition. Among various lines, several inbred lines, which are derivates from selected populations are highly polygenic animal models for body weight regulation, for example the selection line DU6i (Bünger *et al.,* 2001). Selection for high body weight over more than 100 generations from a highly heterogeneous base population in DU6 mice is expected to fix growth promoting QTL alleles, at least those contributing appreciably to the selection response. Hence, selection lines and their inbred derivates offer the possibility of detecting the accumulated QTLs affecting the selected trait.

So far, many QTLs have been identified in the intercrosses DU6 x DUKs and DU6i x DBA/2 (Brockmann *et al.,* 1998, 2000, 2001). The identified QTLs accounted for one third of the phenotypic variance in the examined F_2 populations. Therefore, gene-gene-interaction effects were expected in addition to the direct QTL effects. The F_2 populations DU6i x DBA/2 (Brockmann *et al.,* 2000) and SM/J x LM/J (Cheverud *et al.,* 2001) have been used for the systematic analysis of genome-wide epistatic interaction between QTLs. In both experiments, the pattern of interaction for adiposity and body weight were multilayered. Almost every identified QTL participated in some epistatic interaction. Interestingly, in each of the two populations distinguished QTLs interacted with many different QTLs, these were *Lepq1* on chromosome *14* in the cross DU6i x DBA/2 and *Adip8* at chromosome *18* in the cross SM/J x

LM/J. These findings are very interesting for the identification of nodal points in the complex regulation of the trait.

Different breeding strategies have been developed to elucidate the correlation between genotypes and phenotypes in complex traits (Darvasi, 1998). We are currently developing chromosome substitution strains (CSS) to dissect the genome of the high body weight-selected inbred line DU6i. CSS have a single chromosome from a donor strain substituting for the corresponding chromosome of a recipient strain. The advantages of CSS are summarized by Nadeau *et al.* (2000). In particular, these are, (1) the partitioning of the genome into an ordered subset of genome portions, the chromosome-wise partitioning, (2) the ability to generate as many individuals as needed to prove significant evidence for small effects on a specific chromosome is almost unlimited, (3) the chromosome-wise direct additive and dominant effects of genes located on the substituted chromosome are detectable, (4) the targeted construction of bi-consomic lines is novel and a powerful method for discovering epistasis. A panel of CSS in the mouse consists of 21 strains for 19 autosomes and two sex chromosomes. Here we present data on the generation of these strains.

MATERIAL AND METHODS

Mouse lines. The study was carried out with the mouse line DU6i as donor line and DBA/2 as recipient strain. DU6i has been inbred for 18 generations from the line DU6 which has been selected for high body weight over 100 generations at the age of 6 weeks (Bünger *et al.,* 1990). DU6i mice were twice as heavy as control animals of the selection experiment. Animals were fed *ad lib*. with a breeding diet containing 12.5 MJ/kg metabolic energy with an average content of 22.5% crude protein, 5.0% crude fat, 4.5% N-free extract, vitamins, trace elements, amino acids, and minerals (Diet 1314; Altromin, Lage, Germany).

Experimental design. For the construction of CSS, recurrent backcrossing to a recipient line was used. Initially, one DU6i male was crossed to three DBA/2 females. Subsequently, the F_1 individuals were crossed back to DBA/2 mice. Beginning with the first backcross generation (BC_1) animals harboring a non-recombinant chromosome of interest from line DU6i were selected for subsequent mating to generate the following generation. Repeated backcrosses to strain DBA/2 reduced the portion of the donor genome and increased the portion of the recipient genome.

Markers. The transfers of the chromosome of interest as well as the genetic background of the recipient line were controlled by 148 informative genetic markers. The markers were distributed over all chromosomes, with an average distance of 9.38 cM between markers.

RESULTS AND DISCUSSION

The fifth generation of backcross has been finished for most of the CSS. For the construction of CSS for chromosomes *2*, *4*, *7*, and *19* we are now at the third backcross generation. Table 1 shows the number of animals obtained in every backcross generation and the average portion of the DU6i genome in comparison to the theoretical estimates.

Table 1. Portion of the recipient genome (DBA/2) in animals of different generations

Generation	No of animals	Average percentage of DBA/2 genome (%)	Expected percentage of DBA/2 genome (%)
F1	21	50	50
BC1	157	74.3	75
BC2	368	83.4	87.5
BC3	510	90.3	93.75
BC4	875 [A]	n.d. [A]	96.88
BC5	488 [A]	n.d. [A]	98.44

[A] the generations have not been finished; n.d. not determined

In the generations BC_1 and BC_2, there were animals carrying more then one chromosome of interest. In generation BC_3, 89% of all animals that were selected for subsequent mating had only one chromosome of interest. Therefore, the number of mice that was necessary to transfer the chromosomes of interest to the next generation increased with every following generation. In BC_2 and BC_3, the observed portions of the DBA/2 genome were smaller then the theoretically expected values. This might be a random effect, but, it could also result from higher viability of those animals having a higher portion of DU6i genome. Under optimal condition, it would be possible to produce CSS after five backcross generations. This could be realized if so called "best animals" were selected for the next generation. These are animals, which carry the target chromosome and the biggest portion of the DBA/2 genome on the other chromosomes. However, we could not follow this strategy because we had to take all available animals carrying the chromosome of interest as a parent for the next generation and could not select against DU6i background on the other chromosomes.

The body weight in the original selected inbred line DU6i was 3.6-fold higher than in DBA/2 mice. Corresponding to the increasing portion of the DBA/2 genome and simultaneous reduction of the DU6i genome, the mean body weight was reduced in every cross back to the DBA/2 strain. The changes in body weight are shown in Table 2.

Table 2. Body weight at 42 days in the base lines, the F1, and backcross populations

Population	Body weight at 42 days (mean ± s.e. in g (number of animals))		
	Males and females	Males	Females
DU6i	54.0 ± 4.1 (104)	60.1 ± 3.9 (52)	47.9 ± 4.3 (52)
DBA/2	15.1 ± 1.6 (25)	15.5 ± 1.7 (13)	14.8 ± 1.5 (12)
F1	34.1 ± 4.5 (21)	36. 4 ± 3.0 (15)	28.4 ± 1.9 (6)
BC1	23.2 ± 5.8 (138)	25.8 ± 5.9 (76)	20.3 ± 3.1 (62)
BC2	19.2 ± 4.2 (368)	20.5 ± 4.4 (168)	18.0 ± 3.6 (200)
BC3	17.7 ± 3.3 (510)	18.8 ± 3.5 (270)	17.0 ± 3.0 (240)
BC4	16.7 ± 3.3 (875)	17.5 ± 3.6 (446)	15.8 ± 2.6 (429)
BC5	16.1 ± 2.8 (488)	16.4 ± 3.3 (228)	15.7 ± 2.2 (260)

Already at the third backcross generation, the different CSS differed in the mean body weights. In the generation BC5, the biggest effects on body weight in males were seen in substitution strains for the chromosomes *1*, *7*, *14*, *15*, *16*, and *18*, for females, the biggest effect was found on chromosome *7*. In previous linkage analyses, the biggest effects on body weight and fatness were found on chromosomes *7* and *11* in the cross DBA/2 x DU6i (Brockmann *et al*., 2000). We have not seen significant sex-dependent effects of the QTLs for body weight in the intercross population DBA/2 x DU6i. The genetic effects of substituted chromosomes in the CSS on body weight tend to be different in males and females. For example, the chromosome *7* effect on body weight, was much higher in females as compared to males. The differences in body weight at 42 days between CSS for chromosome *7* and inbred DBA/2 animals were 6.1±2.4 g (n=6) and 4.9±1.9 (n=13) g in males and females, respectively. The phenotypic data have to be confirmed by the analysis of additional animals per strain in advanced backcross generations.

CONCLUSION

The chromosome substitution strains provide valuable resources for future studies of genetic determinants of body weight regulation. The novel sub-strains will likely provide sub-phenotypes, which beside growth and obesity might display distinct effects on phenotypic changes of other traits which have yet to be observed, as for example on fertility, a trait which is easy to record. The chromosome substitution strains are an excellent basis for the subsequent construction of congenic lines to isolate specific QTLs. Furthermore, the search for gene-gene-interaction effects by targeted crosses of CSS and/or congenic strains will provide evidence for interaction effects that have been estimated previously in the F_2 pedigree. We expect significant results for the identification of modifier loci contributing to high growth and obesity and insight into the complex regulation of body weight control.

REFERENCES

Brockmann, G.A., Haley, C.S., Renne, U., Knott, S.A. and Schwerin. M. (1998) *Genetics* **150** : 369-381.

Brockmann, G.A., Kratzsch, J., Haley, C.S., Renne, U., Schwerin, M. and Karle, S. (2000) *Genome Res*. **10** : 1941-1957.

Brockmann, G.A., Haley, C.S., Wolf, E., Karle, S., Kratzsch, J., Renne, U., Schwerin, M. and Hoeflich A. (2001) *FASEB J*. **15** : 978-987.

Bünger, L., Herrendörfer, G. and Renne U. (1990) *Proc. 4th WCGAP* **23** : 321-324.

Bünger, L., Laidlaw, A., Bulfield, G., Eisen. E.J., Medrano. J.F., Bradford, G.E., Pirchner, F., Renne, U., Schlote, W. and Hill, W.G. (2001) *Mamm Genome* **12** : 678-86.

Cheverud, J.M., Vaughn, T.T., Pletscher, L.S., Peripato, A.C., Adams, E.S., Erikson, C.F. and King-Ellison, K.J. (2001) *Mamm Genome* **12** :3-12.

Darvasi, A. (1998) *Nature Genet.* **18** : 19-24.

Nadeau, J.H., Singer, J.B., Matin, A. and Lander, E. S. (2000) *Nature Genet*. **24** : 221-225.

Pérusse, L., Chagnon, Y.C., Weisnagel, S.J., Rankinen, T., Synder, E., Sands, J. and Bouchard, C. (2001) *Obes. Res*. **9** : 135-169.