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# Transfer of brain dopamine system-specific quantitative trait loci onto a C57BL/6ByJ background

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Analysis of complex traits has been difficult because they are usually affected by multiple genes, by the environment, and, importantly, by intra-locus, intergenic, and genotype-environment interactions. To eliminate some of these obstacles and thus provide a tool for identifying specific gene effects in a complex trait, we conceptualized and developed quantitative trait loci (QTL) Introgression (QI) lines and derived Recombinant QTL Introgression (RQI) strains from the QI lines. In genetic background the QI lines are nearly identical; however, differences at one or a few loci are responsible for differences in the phenotype of interest. In addition, QI lines carry passenger genes from the donor genome, which are linked to the differential gene and reside on the same chromosome segment, and whose presence makes chromosome mapping of the differential genes feasible. The mesencephalic activity of tyrosine hydroxylase (TH/MES) was our target phenotype, because it demonstrates significant genetic variation (Ciaranello et al. 1972; Vadasz et al. 1982; Vadasz et al. 1987), because the differences in TH/MES can be attributed entirely to differences in number of dopamine (DA) neurons in the midbrain (Ross et al. 1976; Baker et al. 1980), and because DA systems affect many aspects of behavior. Here we report the development of replicated QI lines with significantly different TH/MES and our preliminary results on behavioral consequences of genetic manipulation of the mesotelencephalic DA system.

Inbred mouse strains with high [BALB/cJ, (C)], low [CXBI/ByJ (I)], and intermediate [C57BL/6ByJ (B6)] TH/MES were obtained from The Jackson Laboratory (Bar Harbor, Maine) at the beginning of this project, in 1983. These animals and their descendants were maintained at the Animal Facility of the Nathan Kline Institute, Orangeburg, NY. In the development of the QI lines the following notation was used: $b_n i_n$ designates the number of backcrosses ($b_n$) and intercrosses ($i_n$), and backcross-intercross cycles were designated $M_n$ (Snell 1948), with $M_1$ corresponding to the $F_1$ and $F_2$ generations, $M_2$ to the first backcross-intercross cycle, including $b_1 i_0$ and $b_1 i_1$ generations, etc. Offspring of $b_5 i_7$ were designated $M_6 F_1$. $F_n$ represents the number of consecutive brother × sister matings after the last backcross-intercross cycle.

TH/MES was determined as described (Vadasz et al. 1987). Activity was expressed as nmol of [$^{14}$C]DOPA formed/MES/h. Intra- and inter-assay variation was controlled by using standardized reference samples. Final TH/MES values were calculated as (3 × corrected TH/MES) ÷ (B6 reference TH/MES). Thus, by definition, TH/MES in B6 was 3.00 nmol of DOPA/MES/h.

To develop QI lines, we used C and I as donor strains, and B6 served as the background strain. $F_2$ generations were derived from mating B6 females to C or to I males. α and β closed replicate lines were created by equal division of each (B6XC)$F_2$ and (B6XI)$F_2$ litter, resulting in four lines: B6.C-α, B6.C-β, B6.I-α, and B6.I-β. The genes responsible for the strain difference in TH/MES were transferred from the partner genome to the background genome by selection for high and low TH/MES with concomitant backcrosses to the background strain. Two replicate lines were created from the high (B6.C-α and B6.C-β) preparation and two from the low (B6.I-α and B6.I-β) preparation. Each line had 15 or more mating pairs, whose 45–90 male offspring were backcrossed to B6 females or intercrossed with non-littermate females of the same generation; the males after having their own offspring were tested for TH/MES. At the age of 219 ± 49 days (mean ± SD, N = 3157), the animals were killed by stunning, followed by quick decapitation and speedy removal of the brain. In each generation, about 1/3 of the males were selected on the

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basis of their TH/MES values (that is, their offspring were used to set up the new mating pairs), and were either backcrossed to B6 females or intercrossed within their lines, strictly avoiding brother-sister mating. In the B6.C and B6.I lines, selection was directed towards the high and low values, respectively. Data for the B6 strain were collected simultaneously with those for the QI lines to detect potential deviations of the developing congenic lines from the background strain.

The first selection was carried out in the $F_2$ generation and was followed by five backcross–intercross cycles. We estimated that after five backcross–intercross cycles the probability of retaining a nonselected, nonlinked donor gene is about 3% [assuming a probability of crossovers between passenger and differential genes of c = 0.5; (Green 1981)]. The last selection was done in the $b_5i_7$ generation. Then, replicate B6.$CM_6$-α, B6.$CM_6$-β, B6.$IM_6$-α, and B6.$IM_6$-β sets of RQI lines were derived from the offspring of the $b_5i_7$ generation. For each set 34 new congenic RQI lines were established by strict brother × sister matings to drive the heterozygous genes into a homozygous condition. In addition, smaller replicate sets of lines, designated B6.$CM_5$-α and B6.$CM_5$-β, were derived from a cross between the $b_3i_5$ generation and the B6 background strain, designated $M_5F_1$. Currently, we are at generations $F_{13-20}$ of 63 B6.$CM_6$ and 55 B6.$IM_6$ RQI strains, but the present report deals with data collected during the development of the QI lines.

A comparison of the population means (±SE) for TH/MES in the $b_5i_7$ QI lines (B6.C-α, 3.49 ± .05; B6.C-β, 3.38 ± .05; B6.I-α, 2.81 ± .03; and B6.I-β, 2.82 ± .02) and in the B6 background strain (3.00 ± .03) indicated (i) that there were significant differences (one-way ANOVA followed by Tukey's post hoc multiple comparison tests: B6.C-α, B6.C-β > B6 > B6.I-α, B6.I-β; HSD alpha = 0.05, df = 272; the value of the minimum significant difference was 0.157), and (ii) that the original deviation of the $F_2$ populations from the B6 background strain was reduced by 25% in the B6.C lines, and by 57% in the B6.I lines. The results suggest that we might have lost some of the differential genes. However, it is also possible that the initial larger differences were due to nontransferable epistatic interactions. Like the mean value deviations, variations in the $F_2$ populations were larger than those in the QI lines. We assume that this is partly due to the fact that in each animal the genetic interactions are different in a segregating $F_2$ generation, leading to higher variability in $F_2$s than in congenic QI lines. In the course of the gene transfer, coefficients of variation (CV) of TH/MES were higher in the B6.C than in the B6.I populations ($F_{(1,43)} = 10.81$; $p < 0.005$); this may indicate that the size of the effect of the differential gene(s) was larger in the B6.C lines. No consistent association could be detected between CV and the type of crossing (that is, backcross or intercross).

To assess the complexity of the genetic control of TH/MES, we reanalyzed power-transformed (p = 0.3) data from the B6XC and BXI crosses, including the parental strains, and $F_1$ and $F_2$ hybrids (Vadasz et al. 1987), by using BCROSS, a computer program of the S.A.G.E. (1992) program package. We tested one-locus, two-locus, and polygenic hypotheses or a mixture of them, using the maximum likelihood method (Elston 1984). For the B6XC cross, the hypothesis that maximized the expected entropy and did not give rise to $\chi^2$ values significant at 5% was the one for two unlinked loci, equal dominance ratio, repulsion. A similar analysis of the BXI cross yielded only hypotheses with significant $\chi^2$ values ($p < 0.05$). We did not test oligogenic hypotheses, because three or four loci begin to approximate the assumptions made for polygenic inheritance. In view of the limitations of such analyses, it seems safe to suggest that a few genes affect TH/MES against a polygenic background and that purely polygenic control is rather unlikely.

To confirm the expectation that the QI lines are congenic at the end of the gene transfer phase, we analyzed allelic variations at polymorphic SSR loci in the $b_5i_7$ lines, the donor strain, and the background strain. PCR reactions were carried out with radioactively labeled primers, and products were analyzed by polyacrylamide gel electrophoresis (Dietrich et al. 1992). One primer pair was selected for each chromosome from a murine genetic map (Dietrich et al. 1992). Primer pairs (MapPair TM) were supplied by Research Genetics (Huntsville, Ala.). DNA was prepared from spleens of male mice by standard phenol–chloroform extraction. Seventeen chromosomes, one locus per chromosome, were tested for polymorphisms. Although the congenic lines were significantly different from the background strain in TH/MES, 212 of the tested 218 SSR genotypes in the congenic animals were of B6 background type, demonstrating that a largely homogeneous genetic background was ensured for the selectively favored TH/MES genes. Three loci, assigned to Chromosomes (Chrs) 10 (*D10Mit10*), 17 (*D17Mit11*), and 18 (*D18Mit17*), were found in heterozygous condition in the B6.C-α QI line. Heterozygosity of loci can result either from randomly distributed nonselected, nonlinked donor genes or from chromosome segment(s) carrying the differential gene. The results suggest that we succeeded in transferring the gene, or genes, that affect the mesotelencephalic dopamine system onto the B6 background. The probability of retaining a nonselected, nonlinked BALB/cJ gene (3%) agrees well with the observed proportion of heterozygotes. A more definitive answer requires high-density, whole-genome mapping of the RQI strains, and correlation analysis between mean TH/MES and the observed genotypes. We predict that clustering of donor type markers and correlation of such markers with TH/MES strain means will reveal the differential chromosome segment(s).

Standard open-field (OF) behavior variables, which were significantly different in the progenitor strains but were not selected for, demonstrated no significant deviation from the background strain after five backcross–intercross cycles (Fig. 1). For example, at the outset of the project, OF locomotor activities in B6, C, and I progenitor strains were 100%, 27.6% and 90.8% respectively (Vadasz et al. 1992a). However, at the end of the $M_6$ cycle locomotor activities in the B6 background and the B6.C and B6.I QI lines were 100%, 99.6%, and 103.6% ($p > 0.05$; data were analyzed by one-way ANOVA with repeated measures followed by Tukey's HSD tests). Similar progressive dissipation of large initial differences was observed in OF defecation, which is considered a marker for emotionality and fear. Lack of significant differences in spontaneous locomotor activity and defecation in the QI

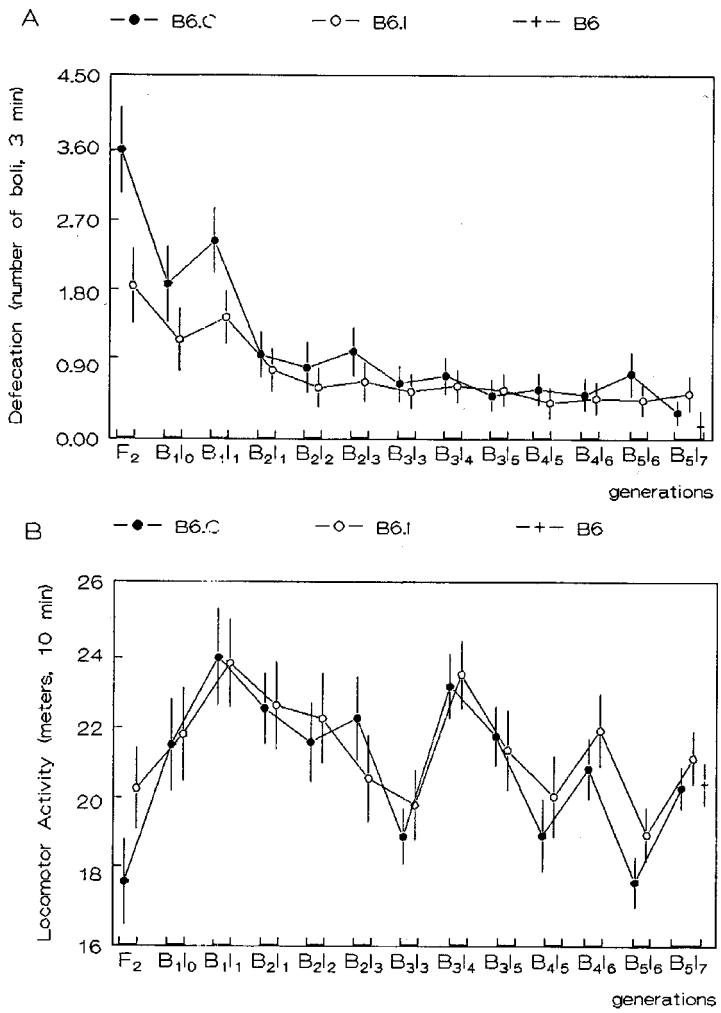


**Fig. 1.** Open-field behavior was recorded by computer-assisted video-tracking in consecutive 1-min intervals for 10 min in our standard open-field environment (Vadasz et al. 1988). (A) Number of feces boli deposited in the first 3 min of the open-field test (mean ± 95% strict confidence intervals) in B6.C and B6.I QI lines and their B6 background strain. (B) Locomotor activities (distance covered, m) in 10 min of the open-field test (mean ± 95% strict confidence intervals) in B6.C and B6.I QI lines and their B6 background strain.

lines suggests that alleles responsible for the original strain differences were replaced by B6 alleles during the gene transfer phase, and consequently TH/MES and these OF behaviors were spuriously associated in the C and I donor strains. Moreover, this confirms the results of our previous multivariate genetic analysis suggesting that neither mesencephalic nor striatal TH activity was predictive of spontaneous locomotor activity in our standard OF test conditions (Vadasz et al. 1992b).

The RQI principle can be instrumental in the study of effects of individual QTLs and in mapping and positional cloning of genes that affect a complex trait. SSR markers show about 50% polymorphism in a typical cross between inbred strains (Dietrich et al. 1992) and are currently spaced less than 1 cM apart (Copeland et al. 1993). In comparison, the average length of differential chromosome segments after five backcrosses is about 33 cM (Flaherty 1981). Therefore, with SSR markers and an RQI system, it will be feasible to locate chromosome segments that carry the relevant QTLs.

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