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Macronutrient choice of BTBR.NZW mice congenic for a 21-gene region of chromosome 17

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ABSTRACT

There has been scant work to investigate the mechanisms influencing macronutrient selection by mice. Here, we measured the consumption and choice of carbohydrate- and fat-containing diets by NZW/LacJ (NZW) and BTBR/T$^+$ *tf*/J (BTBR) strains. We found that NZW mice voluntarily ate more carbohydrate and less fat than did BTBR mice. Mice with a BTBR background and a heterozygous (BTBR/NZW) congenic region on chromosome 17 between 25.7 and 27.5 Mb (N_{10} generation) or 26.7 and 27.5 Mb (N_{12} generation) also ate more carbohydrate and less fat than did homozygous (BTBR/BTBR) littermate controls. Of the 21 known and predicted genes in the congenic interval between 26.7 and 27.5 Mb, we raise for consideration as a causative candidate *Itpr3*, the inositol triphosphate receptor type 3 gene, which is a component of the GPCR-mediated taste transduction cascade. We speculate that a mutation in *Itpr3* influences food choice by impairing the detection of nutrients in the macronutrient-containing diets.

1. Introduction

It would be useful to know which genes influence what we eat. Complex interindividual genetic variation and wide-ranging environments render this difficult to investigate in humans (reviews [1,2]) but genes and environment can be controlled more rigorously in mice. Despite this, there has been only one concerted effort to discover the genes responsible for the macronutrient choice of mice. Smith et al. [3] found that C57BL/6J (B6) mice had moderate carbohydrate preferences whereas CAST/Ei (CAST) mice had the highest carbohydrate preferences of 13 strains tested. Based on a B6×CAST F_2 intercross, Smith Richards et al. [4] discovered six quantitative trait loci (QTLs) linked to three interrelated measures of macronutrient preference. Strong linkage was found to a region of chromosome (Chr) 17, with a peak at ~26 Mb (10 cM) and a 1.5-LOD confidence interval between 0 and ~48 Mb (0–24 cM). This QTL, named *Mnic1* for "macronutrient intake (carbohydrate) 1", was introgressed into a B6.CAST congenic line, with the congenic interval between 0 and 66 Mb on Chr 17 [5]. When given a choice between two diets that contained adequate protein and micronutrients but that differed in carbohydrate and fat, the congenic mice ate more carbohydrate than did wild-type controls [5].

This congenic interval contains more than 1000 genes of which three were considered strong candidates to underlie the phenotypic variation: *Clps*, *Glo1* and *Glp1r*. In later work, Kumar and Smith Richards [6] used gene expression microarrays of liver, skeletal muscle and hypothalamus to identify dozens more candidate genes. Kumar et al. [7] then produced a subcongenic line with introgressed material restricted to ~854 genes between 4.8 and 45.4 Mb, and they found 36 genes differentially expressed in skeletal muscle and 35 genes differentially expressed in hypothalamus. Progress now appears to be thwarted by the large number of candidate genes in the congenic interval.

Based on an F_2 intercross of BTBR T$^+$ *tf*/J (BTBR) x NZW/LacJ (NZW) mice, we recently identified a region of chromosome 17 with a peak at 27.6 Mb that is linked to preferences for several taste compounds [8]. The linkage to saccharin taste preference is extremely strong (LOD > 100) and accounts for 31% of the phenotypic variance, with the NZW haplotype being dominant. Over 10 backcross generations, we introgressed this QTL into a 1.8-Mb region of Chr 17 between 25.7 and 27.5 Mb, near the peak of *Mnic1* (Fig. 1). A recombination occurring in the 11th backcross generation of this line reduced the congenic interval to 0.8 Mb (26.7–27.5 Mb). Given that genetic variation in this region influences taste preferences, and taste preferences influence food choice, it seemed reasonable to evaluate whether these new congenic mice differed from controls in macronutrient choice.

2. Methods

2.1. Design

We used methods similar to those described by Smith Richards et al. [4] to compare the macronutrient choice of (a) inbred NZW mice with inbred BTBR mice, (b) Chr 17 BTBR.NZW-(*rs33353198-rs3656446*)/Mon congenic mice (heterozygous BTBR/NZW) with littermate controls (homozygous BTBR/BTBR), and (c) Chr 17 BTBR.NZW-(*rs47196150-*

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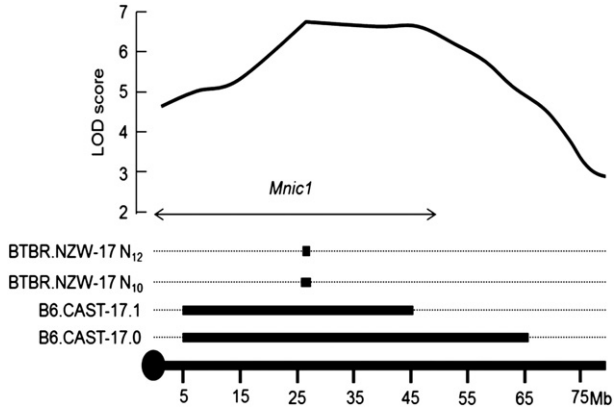

Fig. 1. Chromosome 17: Positions of the *Mnic1* [Macronutrient intake (carbohydrate) 1] QTL and the congenic intervals of three strains of mice. Graph at top of figure shows interval map of carbohydrate intake linkage, from Ref. [4] adapted imprecisely because data are converted from centimorgans to megabases. Arrows show 1.5-LOD confidence intervals of *Mnic1*. Horizontal bars show intervals of congenic strains: BTBR.NZW-17 N_{12} and BTBR.NZW-17 N_{10} = the line of mice described here in the 10th and 12th generations. B6.CAST-17.0 = strain described in Ref. [5]; B6.CAST-17.1 = strain described in Ref. [6].

rs3656446)/Mon congenic mice with littermate controls. The congenic mice and their littermates were members of the 10th or 12th backcross generations, respectively, of the same line and so we refer to them more conveniently as N_{10} and N_{12} congenics. Due to difficulties with animal supply and equipment shortages, separate experiments were conducted using male and female mice. Thus, we conducted a total of six experiments, involving the groups of mice listed in Table 1. All procedures were approved by the Monell Chemical Senses Center Animal Care and Use Committee.

2.2. Subjects

The NZW and BTBR inbred mice were bred in our vivarium from stock purchased from The Jackson Laboratory (strain numbers 001058 and 002282). Generation of the Chr 17 congenic line was accomplished using the following methods: BTBR × NZW F_1 mice were repeatedly backcrossed to the BTBR strain, with each generation containing 44–154 mice. Mice heterozygous (i.e., BTBR/NZW) at *rs3693494* on Chr 17 were used as parents for the first backcross generation. As backcrossing progressed, additional SNPs were genotyped to localize the regions where recombination occurred. Genotyping was accomplished using ABI Assay-by-Design kits (Applied Biosystems, Foster City, CA). Recombinations that narrowed the congenic interval occurred in the N_4, N_6 and N_8 generations, and the mice with recombinations were used to start new lines. The congenic interval in the N_{10} generation was bounded by a recombination between *rs3353198* and *rs45734497* (25.65–26.12 Mb; all locations refer to NCBI Build 37) proximally, and *rs33071006* and *rs3656446* (27.26–27.48 Mb) distally (Fig. 1). The interval in the N_{12} generation was bounded by a recombination between *rs47196150* and *rs33434357* (26.66–26.70 Mb) proximally and the same markers as for the N_{10} generation distally.

2.3. Test procedures

The mice were housed in a vivarium maintained at 23 °C with a 12:12 h light/dark cycle (lights off at 1900 h). They were raised in groups of the same sex until 8–15 wk old. Beginning 7 days before testing, they were individually housed in plastic "tub" cages (dimensions, 26.5 × 17 × 12 cm) with 5–10 mm pine shavings on the floor. Deionized water was available to drink from a 300-mL glass bottle with a stainless steel sipper tube, and pelleted AIN-76A diet (Dyets Inc, Bethlehem, PA; catalogue no. 100000) was available to eat from a hopper built into the cage lid.

At the start of the 8-day test, each mouse was housed in a new cage with two glass jars (30-mL capacity; Fisherbrand, catalogue no. 02-911-912) holding the diets described in Table 2. The distinctive feature of the CHO–P diet was that it contained corn starch and powdered sucrose, whereas the Fat-P diet contained vegetable shortening. Both diets contained casein (protein), minerals and vitamins. The diets were placed in the cage, with the CHO–P diet on the left and the Fat-P diet on the right. To prevent the two jars being knocked over, each was held upright in the center of a 3″ diameter acrylic disk (U.S. Plastics Corp., catalogue no. 44185) by three clear 8-32″ × 7/8″ screw fasteners (U.S. Plastics Corp., catalogue no. 32016). Food spillage using these jars was minimal but any spillage was easily collected from the acrylic disk and was accounted for. In addition, the cage had a corrugated cardboard sheet on the floor (instead of pine shavings) to allow detection and collection of any far-flung spillage. Every 24 h, the food jars and spillage were weighed with 0.1-g precision and the positions of the two jars were switched. In order to maintain freshness, the Fat-P diet was replaced every other day and refilled on alternate days; the CHO–P diet was refilled as needed. Body weights were measured daily to the nearest 0.1 g.

2.4. Data analysis

Preference for the CHO–P diet was determined in two ways: Preferences by weight were calculated as the ratio of CHO–P intake (in g) divided by total intake [in g; i.e., CHO–P intake/(CHO–P intake + Fat-P intake) × 100]. Preferences by energy were calculated using the same formula after weights (in g) were converted to kilocalories based on an energy density of 3.41 kcal/g for the CHO–P diet and 5.95 kcal/g for the Fat-P diet.

Intakes of each diet (in g and in kcal), total intakes (in g and in kcal), and Fat-P diet preferences were analyzed using mixed-design ANOVAs with factors of Strain or Genotype Group and Day. Sex was included as a factor in initial analyses but the pattern of results was the same for both sexes, and sex-related main effects and interactions were small or absent, with the exception of those influencing body weight. Consequently, the intakes of both sexes were combined for

Table 1
Summary of number, age, weight and weight gain of mice tested.

Strain	Male				Female			
	n	Age, days	Body weight, g	Weight gain, g/8 days	n	Age, days	Body weight, g	Weight gain, g/8 days
BTBR	11	108 ± 3	39 ± 1	2.0 ± 0.4	11	110 ± 9	30 ± 1	2.0 ± 0.4
NZW	11	93 ± 15	30 ± 0**	2.1 ± 0.3	12	106 ± 1	25 ± 0*	2.4 ± 0.4
N_{10} congenics (BTBR/NZW)	11	117 ± 11	39 ± 2	2.7 ± 0.6	8	59 ± 2	26 ± 1	2.7 ± 0.2
N_{10} littermates (BTBR/BTBR)	12	123 ± 10	38 ± 1	2.5 ± 0.4	10	61 ± 1	25 ± 1	2.1 ± 0.5
N_{12} congenics (BTBR/NZW)	9	109 ± 9	35 ± 2	3.4 ± 0.9	7	88 ± 2	28 ± 1	4.1 ± 0.6
N_{12} littermates (BTBR/BTBR)	9	95 ± 7	34 ± 1	2.7 ± 0.3	7	88 ± 2	29 ± 1	3.2 ± 0.7

Notes: Values in body of table are means ± SEs. Age = age at the start of the choice test. Weight gain = increase in weight over 8-day two-cup macronutrient choice test. *$p < 0.05$, **$p < 0.01$, relative to BTBR strain. N_{10} congenics had a 1.8-Mb introgressed region between *rs3353198* (25.65 Mb) and *rs3656446* (27.48 Mb) on Chr 17. N_{12} congenics had a 0.8-Mb introgressed region between *rs33434357* (26.70 Mb) and *rs3656446* (27.48 Mb) on Chr 17.