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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⁺ *tflJ* (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 – 27.5 Mb (N₁₀ generation) or 26.7 – 27.5 Mb (N₁₂ 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 – 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.

Keywords

Carbohydrate intake; fat intake; diet selection; taste preferences

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 F₂ 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 – ~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 – 66 Mb on Chr 17 (5). When given a choice between two diets that

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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 >1,000 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 – 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 F₂ intercross of BTBR T⁺ *tf/J* (BTBR) × 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 – 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-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 N₁₀ and N₁₂ 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 F₁ 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 N₄, N₆ and N₈ generations, and the mice with recombinations were used to start new lines. The congenic interval in the N₁₀ generation was bounded by a recombination between *rs33353198* 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 N₁₂ generation was bounded by a

recombination between *rs47196150* and *rs33434357* (26.66 – 26.70 Mb) proximally and the same markers as for the N₁₀ 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 the analyses presented here. Similarly, initial analyses were conducted separately on the N₁₀ and N₁₂ congenic generations, each with its own littermate controls. However, mean values of the two control groups were almost identical so here we compared the results of N₁₀ congenics and N₁₂ congenics with those of the two control groups combined. Post hoc Tukey's tests were used to compare intakes of the groups on individual days when the Group × Day interaction was significant. The criterion for statistical significance was $p < 0.05$.

3. Results

3.1. NZW and BTBR inbred mice

3.1.1. Body weight—At the start of testing, male BTBR mice weighed significantly more than did male NZW mice, $t(20) = 11.3$, $p < 0.0001$; Table 1, and female BTBR mice weighed significantly more than did female NZW mice, $t(21) = 3.58$, $p = 0.0017$. There were no significant differences between the strains in weight gain during the 8-day test (Table 1).

3.1.2. Macronutrient diet intakes—NZW mice ate more CHO-P diet than did BTBR mice, $F(1,43) = 17.2$, $p = 0.0002$. Conversely, BTBR mice ate more Fat-P diet than did NZW mice, $F(1,43) = 107.3$, $p < 0.0001$ (Table 3, Figure 2). The strain difference in CHO-P diet intake was largest on Day 1 and decreased progressively over the 8-day experiment: It was significant on Days 1–6 but not on Days 7–8 [Strain $\times$ Day interaction, $F(7,301) = 10.5$, $p < 0.0001$; Figure 2]. The strain difference in Fat-P diet intake was present on all 8 days, although small day-to-day fluctuations in Fat-P diet intake by the BTBR strain led to a significant Strain $\times$ Day interaction, $F(7,301) = 11.0$, $p < 0.0001$ (Figure 2).

There were no differences between the two strains in the total weight of food consumed over the entire 8-day test, $F(1,43) = 3.20$, $p = 0.0806$, although NZW mice ate more than did BTBR mice on Day 1 [Strain $\times$ Day interaction, $F(7,301) = 9.38$, $p < 0.0001$; data not shown]. However, because Fat-P diet is more energy-dense than CHO-P diet (5.95 vs. 3.41 kcal/g), and BTBR mice ate more Fat-P diet than did NZW mice, energy intake was higher in BTBR than NZW mice, $F(1,43) = 75.6$, $p < 0.0001$ (Table 3, Figure 2), particularly on Day 1 [Strain $\times$ Day interaction, $F(7,301) = 9.37$, $p < 0.0001$; Figure 2].

Preferences of the BTBR males for Fat-P diet were high and remarkably stable throughout the 8-day test (Figure 2). Preferences of the NZW mice were significantly lower [preferences by weight, $F(1,43) = 37.4$, $p < 0.0001$; preferences by energy, $F(1,43) = 35.6$, $p < 0.0001$]. The strain difference was largest on Day 1 and diminished progressively, although the NZW Fat-P diet preferences were always significantly lower than those of the BTBR strain [Strain $\times$ Day interactions, preferences by weight, $F(7,301) = 5.94$, $p < 0.0001$; preferences by energy, $F(7,301) = 8.08$, $p < 0.0001$; Figure 2].

3.2 Congenic mice

3.2.1. Body weight—There were no significant differences in body weights between mice with the BTBR/NZW genotype on Chr 17 and their littermate controls of the same sex (Table 1). There were also no differences in weight gain over the 8-day test.

3.2.2. Macronutrient diet intakes—The two BTBR/NZW congenic groups ate significantly more CHO-P diet and significantly less Fat-P diet over the 8-day test than did the BTBR/BTBR littermate control group [CHO-P intake; $F(2,69) = 6.72$, $p = 0.0022$; Fat-P intake, $F(2,69) = 10.2$, $p = 0.0001$ (Table 3, Figure 3)]. The two BTBR/NZW groups did not differ from each other, except on the first day of the test, when the N₁₂ congenics ate more CHO-P diet than did the N₁₀ congenics [CHO-P intake, Group $\times$ Day interaction, $F(14,483) = 2.72$, $p = 0.0007$]. There were no differences among the three groups in daily energy intake over the 8-day test (Table 3, Figure 3). A significant Group $\times$ Day interaction influencing total energy intakes was present, $F(14,483) = 2.19$, $p = 0.0075$, but post hoc tests could not detect differences in energy intake among the three groups on any day. Instead, there were several differences in energy intakes within groups on different days (for example, the control group ate significantly more energy on Day 2, 3, 4 and 5 than on Day 8).

Preferences of the three groups for the Fat-P diet showed the same pattern of results, whether calculated based on grams consumed or energy consumed: Over the 8-day test, the control group had significantly higher preferences for the Fat-P diet than did either of the congenic groups [preferences by weight, $F(2,69) = 7.13$, $p = 0.0015$, preferences by energy, $F(2,69) = 8.12$, $p = 0.0007$; Table 3, Figure 3]. The congenic groups did not differ from each other, except on the first test day, when the N₁₀ congenics had significantly lower Fat-P preferences than did the N₁₂ congenics, and significantly lower Fat-P preferences than on the remaining 7 days of the 8-day test [Group $\times$ Day interaction, by weight, $F(14,483) = 1.87$, $p = 0.0273$; by energy, $F(14,483) = 2.16$, $p = 0.0085$].

4. Discussion

We found large and persistent differences in macronutrient choice between BTBR and NZW mice, and almost as large and persistent differences between Chr 17 BTBR.NZW- (*rs33353198-rs3656446*)/Mon or Chr 17 BTBR.NZW- (*rs47196150-rs3656446*)/Mon congenic mice and their littermate controls. With the exception of a difference in CHO-P diet intake on the first day of access, the response of mice with the two congenic intervals was remarkably similar. This implies that genetic variation within the smaller congenic region, between 26.66 – 27.48 Mb on Chr 17, underlies the difference in phenotype. The allele inherited from the NZW donor strain is dominant and confers higher carbohydrate intakes, lower fat intakes and lower fat preferences, without affecting total energy intakes.

According to the NCBI database, the *rs47196150-rs3656446* congenic interval contains 14 known genes (Table 4) and 7 unknown (predicted) genes. The congenic interval excludes *Clps*, *Glo1* and *Glp1r*, the three genes raised as candidates in initial work by Smith Richards et al (4,5) and also the 8 candidates that Kumar et al (7) validated as being differentially expressed (*Acat2*, *Agpat1*, *Cyp4f15*, *Glo1*, *Npw*, *Pde9a*, *Pla2gl*, *Ppard*). Consequently, if the same genetic locus is responsible for the macronutrient choice phenotype observed here and by Smith Richards and colleagues then these candidate genes can be eliminated. Two genes present in our congenic interval are also differentially expressed in the B6.CAST subcongenic line and controls (7): *Atp6v0e* in skeletal muscle and hypothalamus and *Bak1* in skeletal muscle. Thus, these two genes may be worthy of further investigation although there is little encouragement from the literature: *Atp6v0e* has no known function (OMIM entry 603931) and *Bak1* is involved in apoptosis (OMIM entry 600516), which seems unlikely to underlie macronutrient selection.

Kumar et al used differential gene expression in skeletal muscle, liver and hypothalamus to identify dozens of candidate genes in the *Mnic1* confidence interval, but a limitation of this approach is that it assumes the analyzed tissues are the appropriate ones to study. We suspect that in this case taste tissue might be more illustrative because our congenic strain has altered taste preferences (8). A prime candidate located in the congenic interval is *Itpr3*, the inositol 1,4,5-triphosphate receptor type 3 gene. ITPR3 has a key role as a component of the cascade of intracellular events involved in taste transduction: It stimulates the release of calcium from the endoplasmic reticulum in response to GPCR-stimulated activation of phospholipase C β 2 [see (9–11)]. Knockout of *Itpr3* leads to loss of sweet, umami and bitter taste detection (12). Although it has not been tested directly, *Itpr3* most likely mediates fatty acid detection as well because this requires *Trpm5*, which is a component of the same transduction cascade as *Itpr3* [(13) review (14)]. A fundamental difference between the CHO-P and Fat-P diets used here is that the CHO-P diet contains sucrose, the prime exemplar of a sweet taste compound, and the Fat-P diet contains vegetable shortening, a source of fatty acids. Thus, we speculate that variation in *Itpr3* is the source of the *Mnic1* macronutrient intake QTL on Chr 17. Public databases show no SNPs that are likely to lead to differences between the BTBR and NZW strains in ITPR3 structure but we have recently

discovered a 12-bp deletion in exon 23 of the BTBR form (but not NZW form) of *Itpr3* (Chr17:27238069). There are also at least 10 SNPs between the B6 and CAST strains in exons of *Itpr3*, including one nonsynonymous SNP in exon 20 (Mouse Phenome Database, 2011). Thus pertinent genetic polymorphisms exist but it remains to be seen whether they have pertinent functional consequences.

The locus for macronutrient choice we have found is based on a BTBR $\times$ NZW cross and so it may not be the same locus as that discovered in the B6 $\times$ CAST cross by Smith Richards et al (4). Indeed, there are differences in the specific phenotype: whereas our congenic mice differ from controls in both carbohydrate and fat intake but not total energy intake, the B6.CAST congenic mice differ from controls in carbohydrate intake and total energy intake, but not fat intake. The four strains involved are from phylogenetically divergent groups (15,16) so it is unlikely that they share a common allele that could be responsible for the phenotype. The only reason to believe the same locus is involved is the coincidence that the BTBR.NZW congenic interval falls close to the peak of the QTL derived from the B6 $\times$ CAST cross (Figure 1). Smith Richards et al (4) found only six QTLs related to macronutrient choice in a whole-genome screen, which gives reason to suspect that macronutrient choice QTLs are relatively sparse, and consequently the probability of two QTLs being located in close proximity is relatively low. Nevertheless, it remains possible that two or more loci in this region of chromosome 17 influence macronutrient selection, and that we have discovered a new QTL.

Smith Richards et al (4) reported energy-based preference scores for Fat-P diet of 53% for B6 mice and 23% for CAST mice. Thus, our NZW mice with 60% Fat-P preference, and BTBR/NZW congenic mice, with 58% or 61% Fat-P preference, are similar to the B6 strain; our BTBR/BTBR group and the BTBR inbred strain, with 73% and 85% Fat-P preference, respectively, are opposite to the CAST strain. The BTBR/BTBR mice, which must have a very similar genetic makeup to the BTBR parental strain after 10 or 12 backcrosses, appear to be a useful model of high fat preference although, if our speculations are correct, this may be less due to their motivation for fat *per se* than to their inability to taste it. It has been argued that reduced sensitivity to the taste of fat leads to obesity in rodents (17) but in this case there was no simple relationship between fat intake and body weight: The congenic mice and their controls did not differ in body weight, daily energy intake, or weight gain during the period they had access to a diet choice.

It seems likely that the gene or genes responsible for the phenotype are located between 26.66 – 27.48 Mb on Chr 17 because the congenic mice with this region introgressed had a virtually identical phenotype to those with the larger region (25.65 – 27.48 Mb). The only significant difference between the two congenic groups involved differences in consumption of CHO-P diet on the first day of the 8-day test. Unlike the N₁₀ congenics and BTBR/BTBR controls, the N₁₂ congenics displayed an initial avidity for CHO-P diet that dissipated after the first day. This is unlikely to be spurious because it was observed in both experiments conducted with these mice (i.e., with males and females). Moreover, a similar response was also observed in NZW inbred mice, and in some of the congenic mice tested by Smith Richards et al (2002). In this wider context, it is highly unlikely that genes captured in the N₁₀ but not N₁₂ congenic mice account for the difference. Instead, we suspect that a latent environmental variable is involved but its identity is a mystery.

In summary, we have determined that genetic variation within a 21-gene region of chromosome 17 influences the macronutrient intakes of mice. We suspect that this locus is responsible for *Mnic1*, a QTL for carbohydrate selection that was discovered based on work with a B6 $\times$ CAST intercross (5). If so, our finding eliminates several previously suggested candidate genes and hones in on others. We suggest that the gene *Itpr3* underlies *Mnic1*, and

that this controls macronutrient selection by influencing the mouse's taste perception of the diets. To confirm this will require additional work, for example, by measuring the macronutrient choice of *Itpr3* knockout mice. It would be informative to know if the B6.CAST congenics have abnormal taste preference phenotypes in addition to their abnormal carbohydrate intake phenotype. Perhaps the *Mnic1* locus will involve taste perception as an endophenotype of macronutrient selection, which is undoubtedly the more complex of the traits.

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Highlights

- NZW/LacJ mice eat more carbohydrate and less fat than do BTBR T⁺ *tf/J* mice.
- A congenic line with BTBR/NZW introgressed region on chromosome 17 was generated.
- The congenic mice behave like NZW mice; thus this region confers diet preference.
- The introgressed region contains 21 genes of which *Itpr3* is a strong candidate.
- Perhaps a mutation in *Itpr3* influences nutrient choice by disrupting taste perception.

Figure 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 N₁₂ and BTBR.NZW-17 N₁₀ = 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).

Figure 2.

Daily intakes and preferences of BTBR mice (n = 22) and NZW mice (n = 23) given a choice between composite diets containing predominantly carbohydrate and protein (CHO-P) or fat and protein (Fat-P) for 8 days. *p<0.05 relative to BTBR strain.

Figure 3.

Daily consumption by chromosome 17 congenic mice given a choice between composite diets containing predominantly carbohydrate and protein (CHO-P) or fat and protein (Fat-P) for 8 days. N₁₀ BTBR/NZW congenics had 1.8-Mb region of NZW strain introgressed between *rs3353198* (25.65 Mb) and *rs3656446* (27.48 Mb) on Chr 17. N₁₂ BTBR/NZW congenics had 0.8-Mb region of NZW strain introgressed between *rs47196150* (26.66 Mb) and *rs3656446* (27.48 Mb) on Chr 17. *p < 0.05 relative to BTBR/BTBR littermate control mice.

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. N10 congenics had a 1.8-Mb introgressed region between *rs3353198* (25.65 Mb) and *rs3656446* (27.48 Mb) on Chr 17. N12 congenics had a 0.8-Mb introgressed region between *rs33434357* (26.70 Mb) and *rs3656446* (27.48 Mb) on Chr 17.

^{*} p<0.05,
^{**} p<0.01, relative to BTBR strain.

Table 2

Composition of carbohydrate-and-protein (CHO-P) and fat-and-protein (Fat-P) diets given as a choice to congenic mice

Ingredient	CHO-P diet	Fat-P diet
Casein, g/kg	198.8	327.7
DL-Methionine, g/kg	2.9	4.9
Sucrose, powdered, g/kg	212.4	0.0
Cornstarch, g/kg	496.2	0.0
Primex (vegetable shortening), g/kg	0.0	519.3
Cellulose, g/kg	49.2	76.2
AIN-76A Mineral Mix #200000, g/kg	32.0	53.3
AIN-76A Vitamin Mix #300050, g/kg	10.0	15.3
Choline chloride, g/kg	1.8	3.1
Energy content, kcal/g	3.41	5.95
% protein	20.8	19.7
% CHO	79.2	1.8
% fat	0	78.5

Notes: Values are in g/kg diet. The small percentage of CHO in the Fat-P diet derives from sucrose used as the diluent for the DL-methionine, mineral and vitamin mixes. The diets were prepared by Dyets Inc, Bethlehem, PA (catalogue no. 103259 and 103260).

Table 3

Measures of daily food consumption averaged over the 8-day food choice test

Measure	NZW (n = 23)	BTBR (n = 22)	BTBR/BTBR (n = 38)	N ₁₀ BTBR/NZW (n = 19)	N ₁₂ BTBR/NZW (n = 16)
CHO-P diet intake, g	2.0 ± 0.2	1.0 ± 0.2 *	1.7 ± 0.1	2.3 ± 0.2 †	2.5 ± 0.2 †
Fat-P diet intake, g	1.6 ± 0.1	3.0 ± 0.1 *	2.6 ± 0.1	2.1 ± 0.1 †	1.9 ± 0.1 †
Total food intake, g	3.7 ± 0.1	4.0 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.5 ± 0.2
Fat-P preference by weight, %	48 ± 3	78 ± 3 *	63 ± 3	50 ± 4 †	46 ± 4 †
Total food intake, kcal	16 ± 0	21 ± 0 *	21 ± 0	20 ± 0	20 ± 1
Fat-P preference by energy, %	60 ± 3	85 ± 3 *	73 ± 2	61 ± 4 †	58 ± 4 †

Notes: Values in body of table are means ± SEs of males and females combined. N₁₀ BTBR/NZW congenics had 1.8 Mb region of NZW strain introgressed between *rs3353198* (25.65 Mb) and *rs3656446* (27.48 Mb) on Chr 17. N₁₂ BTBR/NZW congenics had 0.8-Mb region of NZW strain introgressed between *rs47196150* (26.66 Mb) and *rs3656446* (27.48 Mb) on Chr 17.

* p < 0.0001 relative to NZW strain;

† p < 0.01 relative to BTBR/BTBR littermates.

Table 4List of known genes in the congenic interval between *rs47196150* and *rs3656446*

Start	End	Gene	Name
26698471	26792295	<i>Ergic1</i>	endoplasmic reticulum-golgi intermediate compartment
26813363	26836589	<i>Atp6v0e</i>	ATPase, H ⁺ transporting, lysosomal V0
26918024	26929510	<i>Bnip1</i>	BCL2/adenovirus E1B interacting protein 1
26975610	26978510	<i>Nkx2-5</i>	NK2 transcription factor related, locus
27054036	27069524	<i>Kifc1</i>	kinesin family member C1
27070072	27074835	<i>Phf1</i>	PHD finger protein 1
27074918	27076423	<i>Cuta</i>	cutA divalent cation tolerance homolog
27094363	27107590	<i>Syngap1</i>	synaptic Ras GTPase activating protein
27110112	27113150	<i>Zbtb9</i>	zinc finger and BTB domain containing 9
27156755	27165954	<i>Bak1</i>	BCL2-antagonist/killer 1
27110162	27173323	<i>Ggnbp1</i>	gametogenetin binding protein 1
27194249	27259168	<i>Itpr3</i>	inositol 1,4,5-triphosphate receptor 3
27280914	27304709	<i>Ip6k3</i>	inositol hexaphosphate kinase 3
27326702	27340693	<i>Lemd2</i>	LEM domain containing 2

Notes: Start and end refers to bp position on chromosome 17 (NCBI build 37). Table does not include 7 predicted genes in the congenic interval