Four out of eight genes in a mouse chromosome 7 congenic donor region are candidate obesity genes

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Sarahan KA, Fisler JS, Warden CH. Four out of eight genes in a mouse chromosome 7 congenic donor region are candidate obesity genes. Physiol Genomics 43: 1049–1055, 2011. First published July 5, 2011; doi:10.1152/physiolgenomics.00134.2010.—We previously identified a region of mouse chromosome 7 that influences body fat mass in F2 littersmates of congenic × background intercrosses. Current analyses revealed that alleles in the donor region of the subcongenic B6.C-D7Mit318 (318) promoted a twofold increase in adiposity in homozygous lines of 318 compared with background C57BL/6ByJ (B6By) mice. Parent-of-origin effects were discounted through cross-fostering studies and an F1 reciprocal cross. Mapping of the donor region revealed that it has a maximal size of 2.8 Mb (minimum 1.8 Mb) and contains a maximum of eight protein coding genes. Quantitative PCR in whole brain, liver, and gonadal white adipose tissue (GWAT) revealed differential expression between genotypes for three genes in females and two genes in males. Alpha-2,8-sialyltransferase 8B (St8sia2) showed reduced 318 mRNA levels in brain for females and males and in GWAT for females only. Both sexes of 318 mice had reduced Repulsive guidance molecule-a (Rgma) expression in GWAT. In brain, Family with sequence similarity 174 member b (Fam174b) had increased expression in 318 females, whereas Chromodomain helicase DNA binding protein 2 (Chd2-2) had reduced expression in 318 males. No donor region genes were differentially expressed in liver. Sequence analysis of coding exons for all genes in the 318 donor region revealed only one single nucleotide polymorphism that produced a nonsynonymous missense mutation, Gln7Pro, in Fam174b. Our findings highlight the difficulty of using expression and sequence to identify quantitative trait genes underlying obesity even in small genomic regions.

Genetic complexity on mouse chromosome 7; Rgma; St8sia2; Fam174b; Chd2-2

Obesity is a complex phenotype influenced by genes and environment. Although dozens of genes influencing obesity in humans have been identified, their alleles account for only a small percentage of the total variance in obesity predicted to be genetic based on statistical analysis of families and twins (9, 12). Thus, it is possible that rare alleles of additional genes influence obesity in humans. Studies in mice have successfully identified genes that cause human obesity (20).

In the current study, we used a congenic mouse strain to identify several novel genes that may influence obesity. A congenic mouse strain results from crossing two inbred strains followed by at least 10 generations of backcross to one of the parental inbred strains (background strain) with selection for a small region of alleles from the other parental inbred strain (donor strain). Mice resulting from this crossing scheme have DNA with all background strain alleles except for the region selected during each generation of backcross, which contains donor strain alleles. Any phenotypic differences between the congenic strain and the background strain are caused by gene(s) in the donor region.

Previously, we created several subcongens of the B6.C-Tyr® H1b Hbb+/By (B6.C-H1) congenic mouse strain to narrow a genomic region associated with leanness (7). The B6.C-H1 strain has a BALB/cByJ donor region introgressed on a C57BL/6ByJ (B6By) background. Interestingly, one subcongenic strain, marked by the presence of the BALB/cByJ allele of D7Mit318 (318), displayed an emergent obesity phenotype. This obesity phenotype appeared when we studied 318 mice born to homozygous congenic 318 parents. In our previous study, F2 homozygous 318 subcongenic mice were leaner than their homozygous B6By littersmates. Since the previous study utilized heterozygous F1 parents and our current study used homozygous parents, we hypothesized that the differences in the 318 phenotype could be due to the presence of parenteral genetic effects. Several crossing schemes were employed to differentiate between direct genetic effects and parental genetic effects on obesity.

Direct genetic effects result from alleles present in an individual’s genome. Parental genetic effects can be caused either by imprinting or by allele-dependent expression in the mother during gestation or nursing. During these critical time periods, maternal gene expression can change the offspring’s development and/or gene expression regardless of the offspring’s genotype (10, 22, 30).

F1 reciprocal crosses (congenic × B6By and B6By × congenic) were used to test for imprinting. Cross-fostering studies, in which litters from homozygous congenic parents and litters from homozygous B6By background parents were switched at birth, were used to investigate postnatal maternal genetic effects. We examined direct genetic effects by phenotyping mice raised by their natural, homozygous parents. Along with these crossing schemes, gene expression and sequence analysis were used to identify several novel candidate obesity genes.

Materials and Methods

Mouse Husbandry

All mouse protocols were managed according to the guidelines of the American Association for Accreditation of Laboratory Animal Care and were approved by the institutional review board of the University of California-Davis (Davis, CA). The B6.C-D7Mit318 (318) subcongenic strain was created from the B6.C-Tyr® H1b Hbb+/By (B6.C-H1) congenic strain (The Jackson Laboratory, Bar Harbor, ME), a commercially available strain retaining the H1 histocompatibility antigen allele of the BALB/cBy strain on the back-
ground of the C57BL/6ByJ (B6By) strain (7). Homozygous lines of 318 and B6By mice were maintained in our vivarium at the University of California-Davis. Mice were housed in groups of two to five in polycarbonate cages bedded with CareFRESH (Absorption, Ferndale, WA) and maintained under controlled conditions of temperature (21 ± 2°C), humidity (40–70%), and lighting (14 h light, 10 h dark, lights on at 7 AM). Mice (total number 225) were weaned at 3 wk of age and fed with Research Diets AIN-76A (Research Diets, New Brunswick, NJ; 20.8% protein from casein, 67.7% carbohydrate from sucrose, 11.5% fat from corn oil, 3.9 Kcal/g). Food and water were offered ad libitum.

Reciprocal Cross

Congenic 318 and B6By dams were bred to B6By and 318 congenic males, respectively. Thus, F1 progeny had identical genotype but their maternal genotype was either 318 congenic or B6By.

Cross-Fostering Study

Congenic 318 and B6By dams were bred to give birth on the same day. Within 1 day of birth litters were standardized to six pups and were swapped between mothers so that 318 dams raised B6By pups and B6By dams raised 318 pups. As a control, 318 and B6By dams raised their own pups. Foster and control mothers raised their litters through weaning.

Phenotypic Data Recording

At 112–126 days of age (16–18 wk), mice were killed after an overnight fast. After live body weight was recorded, mice were anesthetized with isoflurane. While still anesthetized, anal-nasal length was also recorded. After death by cervical dislocation, whole brain, liver, and gonadal white adipose tissue (GWAT) were dissected and weighed as described (27). Adiposity index (AI) was calculated as the sum of the four fat depots divided by the total body weight and multiplied by 100 to give the percentage of body weight accounted for by the main fat depots. AI and percent body fat by chemical extraction in our previous experiments have been more replicable within each sex × genotype category than Tbp. Therefore, expression levels of each 318 donor region gene was normalized to Gusb. All expression data were analyzed by the 2^−ΔΔCt method.

Statistical Analyses

A general linear model (ANOVA) was used to assess effects of strain, parent of origin, and fostering on body composition or of genotype on gene expression. Data were analyzed with JMP (SAS Institute, Cary, NC). Post hoc comparisons were by Tukey’s honestly significant difference. P values were corrected for multiple testing using a false discovery rate (FDR) method (1) with a significant P value set at ≤ 0.05. Outliers (± 1.5 * interquartile range) for expression data were removed from the data set.

RESULTS

Defining Congenic Border Regions

The 318 congenic, named due to the presence of the BALB/cByJ allele of the D7Mit318 marker in its donor region, spans a maximum of 2.8 MB on chromosome 7 (Fig. 1). The BALB/cByJ donor region was fine mapped by identification of seven novel dinucleotide repeat markers polymorphic between BALB/cByJ and the background strain C57BL/6ByJ (Supple-

1 The online version of this article contains supplemental material.
mental Table S1). These markers revealed that the maximal 318 donor region lies between 79,400,497 and 82,232,169 base pairs on chromosome 7 (Supplemental Table S1). This region contains eight ENSEMBL protein coding genes: multiple C2 domains, transmembrane 2 (Mctp2), Q8BQR4 (also known as AC136516.1, 2310037124Rik, and C12Orf41 homolog), repulsive guidance molecule A precursor (Rgma), chromodomain helicase DNA binding protein 2 (Chd2), family with sequence similarity 174, member b (Fam174b), alpha-2,8-sialyltransferase 8B (St8sia2), solute carrier organic anion transporter family member 3A1 (Slco3a1), and an unnamed gene (Gm10618) (ENSMUSG00000074066) (Fig. 1 and Supplemental Table S2) (http://uswest.ensembl.org/Mus_musculus/Info/Index). Although we included Mctp2 as a positional candidate, this gene is likely to have background strain alleles in the 318 congenic because the D7B6C2 marker for the maximum proximal border of Mctp2. The first marker with confirmed BALB/cByJ genotype in the 318 congenic is D7B6C3 that is >300 Kb distal.

Obesity Phenotypes of the Homozygous 318 Line

Previous phenotyping of male 318 congenic mice from an F2 cross revealed that homozygous congenic mice were leaner than their B6By background littermates (7). In the current work, we examined obesity phenotypes in male and female mice produced from the homozygous line of 318 subcongenics and the homozygous B6By background strain and found an emergent obesity phenotype in the 318 congenic strain.

We tested the hypothesis that obesity in the 318 strain is due to postnatal maternal effects by reciprocally cross-fostering 318 strain pups to background strain B6By mothers and B6By pups to 318 strain mothers. This analysis revealed significant strain effects on obesity, with the 318 congenic mice being more obese than B6By, but no effect of maternal genotype (Fig. 2 and Supplemental Table S3).

**Heterozygous Progeny of Homozygous Parents are Obese Independent of Parent of Origin or Sex of Progeny**

We also examined imprinting and maternal genetic effects on obesity in the 318 congenic using reciprocal F1 crosses. These crosses revealed that inheritance of congenic alleles either from the sire or the dam did not change F1 obesity (Table 1).

**Gene Expression in the Donor Region**

While Mctp2 was associated with human adiposity in one study, none of the other protein coding genes in the 318 donor region have any previously reported connection to obesity (2). Thus, we examined gene expression and protein coding sequence of seven protein coding genes in the 318 donor region with TaqMan assays. The only gene not examined is the unannotated and unnamed AC116472 gene predicted to make a 44-amino acid product from a 719 bp transcript produced by a single exon. We compared expression levels of genes in the 318 and B6By strains in whole brain tissue, liver, and GWAT since they are key tissues that influence obesity.

**Gene expression in brain.** We first measured gene expression of six protein coding genes in whole brain in 318 congenics and B6ByJ background strain male and female mice. Mctp2 was not quantitated in brain. In females, Fam174b and St8sia2 were expressed at significantly higher levels in B6By mice compared with 318 mice: B6By females had a 1.25-fold increase in Fam174b expression and a 1.35-fold increase in St8sia2 expression (Fig. 3). Male 318 mice had a 1.17-fold increase in expression compared with B6By mice (Fig. 3). Slco3a1, Rgma, and Q8BQR4 did not have differential expression between 318 and B6By strains in brain tissue.

**Gene expression in GWAT and liver.** For GWAT and liver, we measured expression of seven genes in both 318 and B6By strains. Mctp2 was included because the novel microsatellite marker (D7B6C2) that marks the maximal proximal border of...
Fam174b (SNP) we uncovered was a Q7P variant in Supplemental Table S4). Since protein coding sequence alleles can influence obesity independent of mRNA sequence, we analyzed coding sequence for all protein coding genes in the 318 donor region. Primers were designed to span the entire coding sequence of each gene (Supplemental Table S4).

The only nonsynonymous single nucleotide polymorphism (SNP) we uncovered was a Q7P variant in Fam174b. The BALB/cByJ allele of Fam174b altered the Q (glutamine) residue to a P (proline) residue at amino acid position 7 in the coding sequence. When analyzed by the Polyphen program (21) the coding mutation in Fam174b was predicted to be benign. However, the SIFT program (17) predicted that the substitution of proline at amino acid position 7 was deleterious. Unfortunately, both SIFT and Polyphen predictions were made with low confidence since very little is known about the Fam174b protein; it has only been characterized as a Riken clone (18). Uniprot predicted that Fam174b is a single-pass type 1 membrane protein (http://www.uniprot.org/). Uniprot identified amino acid 1–27, the region containing our nonsynonymous SNP, as a potential signaling peptide region of the protein.

We also compared our results with the Sanger SNP database (http://www.sanger.ac.uk/resources/mouse/). Using this database, we confirmed the SNP in Fam174b. The Sanger database also revealed a splice site and a nonsynonymous SNP in Q8BQR4. The ENSEMBL database lists QSBQR4 as a validated protein coding gene; however, NCBI considers it to be a pseudogene. RGMA harbored one 5′-untranslated region (UTR) SNP and St8sia2 had 10 SNPs in its 3′-UTR (Table 2).

DISCUSSION

We have demonstrated that BALB/cByJ donor alleles in the 318 congenic strain promote obesity in mice born to homozygous congenic parents. Previous work in our laboratory identified a leanness effect in homozygous congenic mice when they were born to heterozygous parents (7). In search of an explanation for the discrepancy in phenotype among homozygous mice born to different parents, we tested for parent-of-origin genetic effects. This hypothesis was discounted; neither our F1 reciprocal cross nor our cross-fostering study showed parent of origin effects. These results are consistent with previous quantitative trait locus mapping in the B6.C-H1 derived congenic strains that identified loci for maternal genetic effects that are distal to the 318 donor region (3).

Since there was a considerable time lag between our previous work and the experiments presented here, we also examined whether there had been a reduction in donor region size. We genotyped preserved mouse tissues from the previous experiment and those saved during our current work using SNP markers that define our maximal and minimal 318 donor region. We did not find any differences in donor region size. However, between our maximal and minimal donor region borders, there is a considerable gray area in which we have been unable to find polymorphic SNPs.

Four genes exhibit differential mRNA expression between 318 and B6By strains in either brain or GWAT: Rgma, Fam174b, and St8sia2 in females and Rgma and Chd2-2 in males.

Fam174b is the only gene in the 318 donor region that is also a candidate by virtue of a missense mutation in the coding region. Interestingly, while C57BL/6J and C57BL/6ByJ have the Q form of the SNP, all other mouse strains with sequence information available at this locus have the P form of the SNP. In addition to our discovery of the SNP in BALB/cByJ, Ensemble lists 129s1/SvImJ, A/J, and DBA/2J as having the P

Table 1. Parent-of-origin effects on adiposity index in F1 progeny from a reciprocal cross between 318 congenic and C57BL/6ByJ strains

<table>
<thead>
<tr>
<th>Maternal Genotype</th>
<th>n</th>
<th>318 Congenic</th>
<th>n</th>
<th>C57BL/6ByJ</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>13</td>
<td>6.47 (2.13)</td>
<td>5</td>
<td>5.08 (1.94)</td>
<td>0.22</td>
</tr>
<tr>
<td>Males</td>
<td>14</td>
<td>7.49 (2.66)</td>
<td>10</td>
<td>7.52 (1.39)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Data are means (SD) and were analyzed by 1-factor ANOVA with adiposity index as the dependent variable.

Fig. 3. Relative expression in arbitrary units of 318 donor region genes in whole brain homogenate. Quantitation of gene expression was performed using Taqman RT-PCR and normalized to Gusb. Data are means ± SE, n = 8 per group. Data within each sex were analyzed by 1-factor ANOVA. Significance of main effect P values were determined by FDR procedure (1) set at P ≤ 0.05 and indicated by *.
form of the SNP. Furthermore, a search for orthologs of Fam174b in other species revealed that no other species had a glutamine residue at that position in the protein sequence. However, rat, armadillo, macaque, tree shrew, and lesser hedgehog tenrec all have a proline residue at this position. It is not possible to know if these sequences are polymorphic in other species because polymorphism databases are more limited. It is also interesting to note that all mouse strains identified as having a proline at this amino acid position (BALB/cByJ, 129s1/SvImJ, AJ, and DBA/2J) have a greater percentage of body fat according to the Mouse Phenome Database (http://phenome.jax.org). While these data support a functional role for the Fam174b QTP allele, they do not prove that the SNP is causal for obesity in the 318 congenic.

Rgma expression in GWAT demonstrates a significant genotype effect of the donor region genes in both females and males, with relative expression in B6By mice about twice that in the 318 congenic strain. Several investigators have shown that neogenin is a receptor for Rgma. Knockout of neogenin leads to disturbed ganglia formation and a reduced number of enteric neurons and glial cells in the adult gut (14). A role for Rgma and neogenin in neuronal differentiation has also been demonstrated (15). Thus, both GWAT gene expression and effects on gut neurons are consistent with the hypothesis that Rgma has a role in obesity of the 318 congenic strain.

St8sia2 catalyzes a posttranslational modification of neural cell adhesion molecule by polysialic acid. This modification has been shown to be essential for brain development and cellular adhesion (21, 22). Disruption of St8sia2 in mice results in embryonic lethality due to impaired brain and neural tube development (23). Moreover, human studies suggest that St8sia2 may be involved in obesity (24, 25). However, in the 318 congenic, St8sia2 is expressed at similar levels in both congenic and B6ByJ mice, suggesting that it may not contribute significantly to obesity in this model.

Table 2. SNPs in the 318 donor region

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>C57BL/6J</th>
<th>BALB/cJ</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q8BQR4</td>
<td>79,818,826</td>
<td>T</td>
<td>C</td>
<td>splice site</td>
</tr>
<tr>
<td>Q8BQR4</td>
<td>79,819,974</td>
<td>C</td>
<td>T</td>
<td>nonsynonomous coding</td>
</tr>
<tr>
<td>Rgma</td>
<td>80,520,431</td>
<td>T</td>
<td>G</td>
<td>5’ UTR</td>
</tr>
<tr>
<td>Fam174b</td>
<td>80,885,408</td>
<td>A</td>
<td>C</td>
<td>nonsynonomous coding</td>
</tr>
<tr>
<td>St8sia2</td>
<td>81,084,180</td>
<td>G</td>
<td>T</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>St8sia2</td>
<td>81,084,355</td>
<td>G</td>
<td>A</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>St8sia2</td>
<td>81,084,609</td>
<td>G</td>
<td>A</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>St8sia2</td>
<td>81,084,834</td>
<td>C</td>
<td>T</td>
<td>3’ UTR</td>
</tr>
<tr>
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<td>81,085,083</td>
<td>A</td>
<td>G</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>St8sia2</td>
<td>81,085,144</td>
<td>G</td>
<td>T</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>St8sia2</td>
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<td>C</td>
<td>A</td>
<td>3’ UTR</td>
</tr>
<tr>
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<td>81,085,423</td>
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<td>T</td>
<td>3’ UTR</td>
</tr>
<tr>
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<td>C</td>
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</tr>
<tr>
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<td>G</td>
<td>A</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>St8sia2</td>
<td>81,085,827</td>
<td>A</td>
<td>G</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>St8sia2</td>
<td>81,086,496</td>
<td>T</td>
<td>C</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>St8sia2</td>
<td>81,087,149</td>
<td>C</td>
<td>T</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>St8sia2</td>
<td>81,087,517</td>
<td>G</td>
<td>A</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>St8sia2</td>
<td>81,087,566</td>
<td>T</td>
<td>C</td>
<td>3’ UTR</td>
</tr>
</tbody>
</table>

We queried the Sanger database to find additional single nucleotide polymorphisms (SNPs) in our 318 donor region. On this database, we compared C57BL/6J and BALB/cJ strains. In our experiments, our background strain was C57BL/6ByJ and our donor strain was BALB/cByJ. UTR, untranslated region.
dates. Regulatory RNAs are another potential mediator of the obesity phenotype and warrant further study.

In conclusion, we identified a 2.8 Mb region of mouse chromosome 7 in which BALB/cByJ alleles promote increased body weight and adiposity relative to the B6By strain. We identified four genes with statistically significant genotype effects on expression. One of these four genes has a nonconservative missense mutation (Fam174b). Rgma and St8Sia2 both have functional effects on neuronal development that could influence obesity, but knockouts of the Rgma receptor neogenin and St8Sia2 failed to reveal effects on body weight or obesity. Chd2 is associated in both mice and humans with Type 2 diabetes but not with obesity in the mouse. Thus, our studies demonstrate the presence of at least four potential obesity genes in the 318 congenic but cannot definitively identify any one as the most likely candidate.

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GRANTS
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DISCLOSURES
No conflicts of interest (financial or otherwise) are declared by the author(s).

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