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Genetic factors for resistance to diet-induced obesity and associated metabolic traits on mouse chromosome 17

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Abstract

Obesity is associated with increased susceptibility to dyslipidemia, insulin resistance, and hypertension, a combination of traits that comprise the traditional definition of the metabolic syndrome. Recent evidence suggests that obesity is also associated with the development of nonalcoholic fatty liver disease (NAFLD). Despite the high prevalence of obesity and its related conditions, their etiologies and pathophysiology remains unknown. Both genetic and environmental factors contribute to the development of obesity and NAFLD. Previous genetic analysis of high-fat, diet-induced obesity in C57BL/6J (B6) and A/J male mice using a panel of B6-Chr^{A/J}/NaJ chromosome substitution strains (CSSs) demonstrated that 17 CSSs conferred resistance to high-fat, diet-induced obesity. One of these CSS strains, CSS-17, which is homosomal for A/J-derived chromosome 17, was analyzed further and found to be resistant to diet-induced steatosis. In the current study we generated seven congenic strains derived from CCS-17, fed them either a high-fat, simple-carbohydrate (HFSC) or low-fat, simple-carbohydrate (LFSC) diet for 16 weeks and then analyzed body weight and related traits. From this study we identified several quantitative trait loci (QTLs). On a HFSC diet, *Obrq13* protects against diet-induced obesity, steatosis, and elevated fasting insulin and glucose levels. On the LFSC diet, *Obrq13* confers lower hepatic triglycerides, suggesting that this QTL regulates liver triglycerides regardless of diet. *Obrq15* protects against diet-induced obesity and steatosis on the HFSC diet, and *Obrq14* confers increased final body weight and results in steatosis and insulin resistance on the HFSC diet. In addition, on the LFSC diet, *Obrq16* confers decreased hepatic triglycerides and *Obrq17* confers lower plasma triglycerides on the LFSC diet. These congenic strains provide mouse models to identify genes and metabolic pathways that are involved in the development of NAFLD and aspects of diet-induced metabolic syndrome.

Introduction

Obesity, which is defined as increased adipose tissue mass, results from a chronic imbalance between energy intake and energy expenditure (Stunkard 1996; Weiser et al. 1997). The rapid increase in the prevalence of obesity is associated with considerable morbidity and mortality (Flegal et al. 2002). Most notably, obesity is associated with increased susceptibility to dyslipidemia, insulin resistance, and hypertension, a combination of traits that comprise the traditional definition of the metabolic syndrome (Alberti and Zimmet 1998; Balkau and Charles 1999; Reaven 1988).

Recent evidence suggests that obesity is associated with the development of nonalcoholic fatty liver disease (NAFLD), which has been proposed as a new component of the metabolic syndrome (Khashab et al. 2008). NAFLD is characterized by the accumulation of fat droplets in hepatocytes (steatosis) (Hall and Kirsch 2004). NAFLD can progress to a more severe form of liver disease, non-alcoholic steatohepatitis (NASH), which is characterized by hepatocyte injury and inflammation (Ludwig et al. 1980). In the general population, the incidence of NAFLD and NASH is approximately 17–33% and 5.7–17%, respectively (Ground 1982; Hilden et al. 1977; McCullough 2004). Approximately 20% of patients with NASH progress to cirrhosis (Diehl et al. 1988; Lee 1989; Matteoni et al. 1999), which is characterized by fibrosis and liver failure. NAFLD and NASH have become the most common liver diseases in developed countries (Angulo 2002; Browning et al. 2004; Clark and Diehl 2003; Suriawinata and Fiel 2004).

Despite the high prevalence of obesity and related conditions such as NAFLD, their etiologies and pathophysiology remain unknown. Both genetic and environmental factors contribute to the development of obesity, with approximately 80% of variation in body weight due to genetic contributions (Stunkard et al. 1986). However, interpreting the results from genetic studies of obesity has proven to be complex for a variety of reasons, including difficulties in controlling environmental factors in human populations, challenges related to reproducibility of results across populations (Bell et al. 2005), and the large numbers of individuals needed for complex trait studies (Lango and Weedon 2008; Perusse et al. 2005; Rankinen et al. 2006; Zeggini et al. 2007).

Because of the multifaceted nature of human genetic studies of obesity, animal models are proving to be important tools for the genetics studies of diet-induced obesity. In particular, compared with A/J male mice, the C57BL/6J (B6) males are a well-established model for diet-induced obesity and associated metabolic syndrome traits such as insulin resistance, hypertension, and dyslipidemia (Black et al. 1998; Collins et al. 1997; Mills et al. 1993; Surwit et al. 1988, 1995). Genetic analysis of diet-induced obesity in B6 and A/J male mice was performed using a panel of B6-Chr^{A/J}/NaJ chromosome substitution strains (CSSs) (Singer et al. 2004). This CSS panel consisted of 22 inbred strains each containing a different, nonrecombinant homozygous chromosome derived from the A/J strain substituted onto the genetic background of the B6 strain (Nadeau et al. 2000; Singer et al. 2004). Analysis of weight gain on a high-fat diet in the CSSs demonstrated that 17 CSSs conferred resistance to diet-induced obesity (Singer et al. 2004). In the current study we analyzed CSS-17 and generated seven congenic strains derived from CSS-17. The mice were fed either a HFSC or a LFSC diet for 16 weeks and then were analyzed for changes in body weight and related traits. From this study we have shown that CSS-17 is resistant to diet-induced obesity, steatosis, and insulin resistance and have identified four QTLs that regulate diet-induced obesity, steatosis, and insulin resistance after feeding the mice the HFSC diet. We have identified two additional QTLs that regulate hepatic triglycerides and plasma triglycerides after feeding the mice the LFSC diet.

Materials and methods

Husbandry

B6-Chr 17^{A/J}/NaJ CSS (CSS-17) mice were generated and maintained at Case Western Reserve University. C57BL/6J (B6) and A/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were raised in microisolator cages with a 12 h:12 h light:dark cycle. All mice were weaned at 3–4 weeks of age and raised on LabDiet #5010 autoclavable rodent chow (LabDiet, Richmond, IN) *ad libitum* until diet studies were initiated. The LabDiet #5010 derives 12.7% of its kilocalories (kcal) from fat, 58.5% from carbohydrate, and 28.7% from protein.

Congenic strain construction

To generate homozygous congenic strains, CSS-17 was crossed to B6 mice to generate (CSS-17 × B6) F1 mice, which then were backcrossed to either the parental CSS-17 or B6 (N2F1). N2F1 mice with the desired A/J- or B6-derived segments of chromosome 17 were selected by genotyping and used for congenic strain construction. Males and females (backcross generation N3 or greater) that inherited the intact A/J-derived congenic segment (with no detectable recombination) were intercrossed. Then, the offspring were genotyped to identify offspring that were homozygous for the desired segment, which were then intercrossed to maintain the line.

Markers and genotype

To construct the congenic strains, DNA was isolated from the tail tissue as described previously (Miller et al. 1988; Noguchi and Noguchi 1985). PCR was performed using standard touchdown PCR methods with primers obtained from the Mouse Genome Informatics website (<http://www.informatics.jax.org>). The PCR products were separated using 11% polyacrylamide gel electrophoresis and visualized under ultraviolet light with ethidium bromide. The location of each MIT marker was based on build 37 of the mouse genome. The markers are as follows: m1 = *D17Mit113* (12,172,235 bp), m2 = *D17Mit133* (24,994,647 bp), m3 = *D17Mit198* (27,796,140 bp), m4 = *D17Mit11* (41,461,165 bp), m5 = *D17Mit178* (48,845,705 bp), m6 = *D17Mit139* (52,798,656 bp), m7 = *D17Mit20* (57,366,958 bp), m8 = rs13483078 (67,111,530 bp), m9 = *D17Mit39* (74,681,488 bp), m10 = *D17Mit2* (80,977,573 bp), m11 = *D17Mit155* (84,901,028 bp) (Supplementary Table 1). For SNP analysis of rs13483078, the PCR product was digested with restriction enzyme *Nco*I and analyzed on 11% acrylamide gel. The A/J allele contains the restriction site and produces two fragments (131 and 60 bp), and the B6 allele remains uncut producing one fragment (191 bp).

Diet study

At 5 weeks of age, males were introduced to a high-fat, simple-carbohydrate (HFSC) diet (Research Diets D12331, New Brunswick, NJ) or a low-fat, simple-carbohydrate (LFSC) diet (Research Diets D12329) and maintained on the diet for 16 weeks (until 21 weeks of age). The HFSC diet derives 58% of its kilocalories (kcal) from fat (soybean and coconut oil), 25.5% from carbohydrate (sucrose and maltodextrin), and 16.4% from protein (casein). The LFSC diet derives 10.5% of its kcal from fat (soybean and coconut oil), 73.1% from carbohydrate (sucrose and maltodextrin), and 16.4% from protein (casein). Body weight and food intake were measured weekly. Each measurement of food intake represents a 24-h period and is expressed as food intake/kg body weight.

Plasma collection and serology

At 21 weeks of age, mice were fasted overnight (18 h), weighed, and anesthetized with intraperitoneal injection (IP) of 0.8 mg/g Avertin (2,2,2-tribromoethanol in tert-amyl alcohol) (Fisher Scientific, Pittsburgh, PA). Once anesthetized, blood was collected by cardiac puncture. Plasma was isolated using Microtainer plasma separator tubes (Becton Dickinson, Franklin Lakes, NJ). Measurements of cholesterol, free fatty acids, and triglyceride were performed by Veterinary Diagnostic Services (Marshfield Laboratories, Marshfield, WI). Insulin levels were measured with an Ultrasensitive Mouse Insulin ELISA enzyme immunoassay (Mercodia, Winston Salem, NC).

Liver triglycerides

Liver samples were frozen in liquid nitrogen and 100–200 mg of liver were saponified in an equal volume by weight of 3 M KOH/65% ethanol as described by Salmon and Flatt (1985). Glycerol was measured against glycerol standards using a commercially available Triglyceride GPO reagent set (Pointe Scientific, Lincoln Park, MI) as previously described (Buchner et al. 2008).

Glucose tolerance tests—Glucose tolerance tests were performed in a separate group of male mice fed the HFSC diet for 16 weeks. The mice were fasted for 18 h and injected intraperitoneally (IP) with 2 g glucose/kg body weight. Blood was collected from the tail vein, and glucose was measured at 0, 15, 30, 60, and 120 min after glucose injection using an UltraTouch meter (Lifescan, Inc., Milpitas, CA).

Determination of homeostasis model assessment of insulin resistance—The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using glucose and insulin determinations obtained after an overnight fast using the following formula: [fasting glucose (mmol/l) × fasting insulin (μU/ml)]/22.5 (Haluzik et al. 2006; Xia et al. 2002).

Statistical analysis

The “sequential method” for QTL identification compares trait data between pairs of strains while minimizing genetic differences between those strains. Heritable phenotypic differences between these strain pairs define a QTL which is necessarily located within the interval where these two strains genetically differ. The initial comparison is between the host strain (i.e., B6) and the congenic strain with the smallest genetic difference from the host strain. That congenic strain is then compared with a different congenic strain with the smallest genetic difference between those two strains. These sequential comparisons continue until each strain in the panel has been tested (Shao et al. 2008). Genetic differences between strains were calculated as the maximum physical distance between genetic markers flanking the region of sequence difference. Details of the sequential method are found in Shao and Nadeau (Shao et al. 2008). The sequential comparison conducted on congenics derived from CSS-17 is shown in Fig. 1.

Results

Diet-induced obesity resistance in CSS-17 and congenic strains

Previous studies showed that CSS-17 males were resistant to diet-induced obesity relative to the B6 males (Singer et al. 2004). We expanded this study and analyzed CSS-17 on the HFSC diet for 16 weeks and analyzed various metabolic parameters (Supplementary Table 2). To determine the number and location of QTLs that confer resistance to diet-induced obesity on chromosome 17, we generated a panel of seven congenic strains derived from CSS-17 and analyzed body weight in males from each strain fed the HFSC diet for 16 weeks. When the diet study was initiated at 5 weeks of age, no significant differences in body weight were detected in the seven congenic strains, but at the end of the study CSS-17 and several congenic strains, namely, 17C1, 17C2, 17C3, 17C4, and 17C6, weighed significantly less than B6 (Fig. 2a, Supplementary Table 2).

We analyzed the body weight data using a method involving sequential comparisons between two congenic strains (Shao et al. 2008). The strains with the smallest, unique, A/J-derived segments (17C1 and 17C7) were compared with B6. Then, each of these two strains was compared with the congenic strain containing the next longer A/J-derived segment (17C1 vs. 17C2 and separately 17C7 vs. 17C6). The same process was repeated with the following comparisons done separately (17C2 vs. 17C3, 17C3 vs. 17C4, and 17C6 vs. 17C5). Figure 1 provides a schematic of the analysis methods.

Using the sequential method, we found congenic strain 17C1 was resistant to obesity and thus defines *Obrq13* (obesity-resistance QTL 13) (Fig. 3a). In contrast, 17C7 was indistinguishable from B6. The congenic strain 17C6 was lean compared with 17C7, demonstrating that a second QTL (*Obrq15*) maps to a 32-Mb interval in the more distal segment of the A/J-derived chromosome between markers m7 and m10. Further sequential analysis of the obese strain 17C5, compared with the lean strain 17C6, identified a third QTL for which the A/J-derived allele promotes obesity (*Obrq14*, including m5–6). Thus, the congenic strains provided evidence for three obesity-resistance QTLs (*Obrq13–Obrq15*).

To determine if these diet-induced QTLs also affect obesity normal, we analyzed the parental strains, CSS-17, and the congenic strains on the LFSC diet (Fig. 4A). There were no differences in body weight. Thus, *Obrq13* protects against diet-induced obesity on the HFSC diet.

Decreased food consumption does not explain obesity resistance

A possible explanation for decreased body weight is decreased food consumption. To test whether the obesity-resistant congenic strains consumed less food relative to B6, we measured food consumption in males from each strain. The mice were weighed and food intakes were measured over a 24-h period every week. The values for food intake are shown normalized by body weight (Fig. 2b). The normalized data demonstrated that CSS-17 and all congenic strains consumed similar amounts of or more food than B6. Actual food intakes per day per mouse are shown in Supplementary Fig. 1, which indicates that A/J mice consumed less food per day, whereas the congenic strains 17C1 and 17C2 consumed more food per day per mouse than B6. Thus, food consumption differences do not explain the obesity resistance in 17C1 and 17C6.

Improvement of predicted insulin resistance in fasting conditions in congenic mice

We next hypothesized that the QTLs that conferred resistance to diet-induced obesity may also confer resistance to components of the metabolic syndrome such as hyperglycemia and increased fasting insulin levels. We measured fasting plasma glucose and insulin in CSS-17 and in the congenic strains after 16 weeks of HFSC diet consumption and calculated the homeostasis model of insulin resistance (HOMA-IR) (Supplementary Table 2). CSS-17 had lower fasting glucose, lower fasting insulin, and lower HOMA-IR. Congenic strain 17C1, which contains *Obrq13*, also had significantly lower fasting plasma glucose, lower fasting plasma insulin, and lower HOMA-IR (Fig. 3b, c, Supplementary Table 2). In contrast, the fasting plasma glucose, fasting insulin, and HOMA-IR in 17C7, which includes *Obrq15*, was similar to B6. Congenic strain 17C6 had low levels of insulin but was not significantly different than those of 17C7, which may be due to the relatively small number of animals analyzed ($n = 9$). Thus, the A/J-derived segment containing *Obrq13* conferred resistance to increased fasting plasma glucose and resistance to increased fasting insulin levels with the high-fat diet.

Resistance to NAFLD in congenic strains

Because insulin resistance has been associated with the development of steatosis, we tested whether congenic strains that have lower HOMA-IR also confer resistance to steatosis. When compared with B6, CSS-17 and the 17C1 congenic strain, which were obesity-resistant, also had significantly lower hepatic triglyceride levels after 16 weeks of HFSC diet consumption (Fig. 3d, Supplementary Table 2). Sequential analysis of 17C7 showed no differences compared with B6, and 17C6 (which contains *Obrq15*) was not different than 17C7 ($p = 0.09$). This may be due to the large SEM for these strains or it may be that several genes are interacting and thus prevents identification of a QTL. However, sequential analysis of 17C5 compared with 17C6 showed an additional QTL, *Obrq14*, that acts in a similar manner to B6. Thus, resistance to fatty liver mapped to the same segment as obesity resistance (*Obrq13*) and *Obrq14* results in hepatic triglycerides that are similar to B6.

To determine if the effect of these QTLs is diet-specific, we analyzed the parental strains, CSS-17, and the congenic strains on a LFSC diet (Fig. 4b, Supplementary Table 3). Although there were no differences in liver triglycerides between B6 and A/J, 17C1, 17C2, 17C5, 17C6, and 17C7 had lower hepatic triglycerides. Therefore, *Obrq13* (contained within 17C1) results in reduced steatosis regardless of the diet and *Obrq14* (contained within 17C5) is greatly affected by diet.

Cholesterol and triglyceride metabolism in congenic strains

Several traits associated with obesity include increased cholesterol, triglycerides, and plasma free fatty acids (FFA). We tested whether these differed among CSS-17 and the congenic strains. Analyses of fasting plasma cholesterol in the congenic strains after 16 weeks of HFSC diet consumption revealed that while CSS-17 had cholesterol levels similar to those of B6, congenic strain 17C1 had reduced fasting plasma cholesterol (Fig. 3e). Thus, the A/J-derived allele of *Obrq13* confers reduced cholesterol levels in mice on a HFSC diet. The sequential analysis of 17C5 compared with 17C6 identified an additional QTL, *Obrq14*, that confers increased cholesterol levels.

Comparisons of plasma triglycerides in the congenic strains 17C1 and 17C7 relative to B6 revealed that 17C1 had increased plasma triglycerides B6 (Fig. 3f). The sequential comparison of 17C5 and 17C6 identified an A/J-derived allele within *Obrq14* that results in increased plasma triglyceride levels in mice on a HFSC diet. We measured the fasting plasma triglycerides after LFSC diet feeding and found that an A/J-derived allele within *Obrq15* reduces plasma triglycerides and that an additional A/J-derived allele within QTL *Obrq17* increases plasma triglycerides (Fig. 4c).

Measurements of free fatty acids from 17C1 and 17C7 compared with B6 found no significant differences. However, when 17C6 was compared to 17C7, *Obrq15* confers increased FFA. Thus, elevated FFA levels map to the region that defines *Obrq15* (Fig. 3g). Comparison of 17C5 to 17C6 identified a QTL, *Obrq14*, that reduces FFA on the HFSC diet. Because 17C5 contains both *Obrq14* and *Obrq15*, *Obrq14* is epistatic for this effect.

QTLs associated with improved glucose tolerance tests on HFSC diet

We identified *Obrq13* within the A/J-derived sequence in congenic strain 17C1 that results in lower final body weight, lower glucose, lower insulin levels, lower hepatic triglyceride, lower plasma triglycerides, and lower cholesterol levels compared to B6 mice on a HFSC diet. Because insulin resistance and type 2 diabetes are associated with obesity and steatosis (Leite et al. 2009), we measured glucose tolerance with glucose tolerance tests (GTT) in CSS-17 and the congenic strains that contained identified QTLs and compared them to B6 (Fig. 5). B6 males had an increased rate of glucose clearance and an increased HOMA-IR which predicts insulin resistance, whereas CSS-17 and the congenic strain 17C1 had improved glucose clearance and reduced HOMA-IR compared with B6. The congenic strain 17C5 had improved glucose clearance but had increased HOMA-IR that was similar to B6, suggesting impaired glucose tolerance. Further studies are needed to define the role of insulin resistance in the development of steatosis on HFSC diet in these strains and will be the focus of future studies.

Summary of mapping results in CSS-17 congenic strains

A summary of the mapping data is shown in Fig. 6 for HFSC and LFSC diet studies. We have identified *Obrq13*, which is resistant to diet-induced obesity and steatosis and confers normal HOMA-IR; *Obrq15*, which is resistant to diet-induced obesity and steatosis; and *Obrq14*, which develops diet-induced obesity and steatosis and confers elevated HOMA-IR similar to B6. Future studies will define these regions more precisely and identify genes involved in obesity, insulin resistance, and steatosis. In addition, we found A/J-derived alleles within *Obrq16* that reduce hepatic triglycerides on the LFSC diet, while A/J-derived alleles within *Obrq17* decreases plasma triglycerides on the LFSC diet.

Discussion

Obesity resistance in CSS-17

CSS surveys show that multiple individual A/J chromosomes, including A/J-derived chromosome 17, conferred resistance to diet-induced obesity when substituted onto the B6 background (Singer et al. 2004). However, the results of the CSS surveys do not indicate the number, location, or action of QTLs on each chromosome. Using intercross progeny derived from CSS-17 and B6, we detected a single QTL near the centromere of the chromosome (data not shown). In the present study we extended the phenotypic characterization associated with obesity resistance and a congenic strain panel consisting of seven congenic strains derived from CSS-17. These strains were analyzed on a HFSC diet to determine the location of the QTL detected in the CSS-17 intercross and to test whether additional QTLs could be detected using this approach.

Obrq13 confers resistance to several metabolic syndrome traits

Using these congenic strains, we detected an obesity resistance QTL (*Obrq13*) that maps to the same region as the QTL mapped using CSS-17 intercross progeny (data not shown). Additional studies demonstrated that this same region affects hyperglycemia, hypercholesterolemia, and steatosis. Thus, the A/J-derived allele conferred resistance to the metabolic syndrome phenotype that characterizes B6 males when fed a high-fat diet (Collins et al. 2004). In addition, the A/J-derived alleles within *Obrq13* reduce hepatic triglycerides independent of diet since *Obrq13* resulted in lower hepatic triglycerides on both HFSC and LFSC diets.

Obrq13 maps to an approximately 27.8-Mb region (between 0 and 27.8 Mb on chromosome 17) that contains about 435 genes (Mouse Genome Informatics, <http://www.informatics.jax.org/>). At least three obesity-related QTLs have previously been mapped to this region using other inbred strains. Analysis of intercross progeny derived from AKR/J and C57L/J (fed conventional chow) identified an obesity QTL (*Obq4*) that mapped to a 14-cM region near the centromere of chromosome 17 (Taylor and Phillips 1997). The obesity-promoting allele is derived from C57L/J (Taylor and Phillips 1997). Likewise, an intercross between B6 and 129S1/SvImJ detected a QTL (*Obq19*) for body mass index (BMI) in this same region (fed high-fat diet). As in the present study, the B6 allele confers obesity (Ishimori et al. 2004a, b). In addition, analysis of intercross progeny derived from SM/J and NZB/BINJ fed an atherogenic diet identified *Adip18*, a QTL for fat pad weight, in this same region (Stylianou et al. 2006). In the present study, candidate genes contained within *Obrq13* include insulin-like growth factor II receptor (*Igf2r*) (Ong and Dunger 2001), insulin-like growth factor binding protein (*Igfals*), acetyl-coenzyme A acetyl transferase 2 (ACAT2) and ACAT3 (Tomoda and Omura 2007), tubby-like protein 4 (*Tulp4*) (Santagata et al. 2001), and lipase maturation factor 1 (*Lmf1*) (Peterfy et al. 2007). In addition, many uncharacterized genes map to this region. Thus, further studies are needed to fine-map this QTL interval before candidate gene studies can be pursued.

Obrq15 confers resistance to obesity but not all metabolic syndrome-related traits

We detected a second obesity resistance QTL (*Obrq15*) that maps to an approximately 32.1-Mb region of chromosome 17 (between m6 and m11). This QTL was not previously detected in our CSS-17 intercross mapping study highlighting the benefits of our congenic strain analysis (data not shown). In addition to obesity resistance, the A/J-derived allele of *Obrq15* increases FFA levels. The role of *Obrq15* in the development of diet-induced steatosis is not clear. The sequential analysis did not identify an effect of *Obrq15* in the accumulation of hepatic triglycerides in the HFSC diet. This may be the result of the large variance in 17C7 hepatic triglyceride levels or there may be epistatic interactions. Thus,

unlike *Obrq13*, *Obrq15* affects a subset of the metabolic syndrome phenotypes on a HFSC diet.

The *Obrq15* candidate region contains approximately 244 genes. An obesity-related QTL, *Obwq4*, was previously mapped to this region using the same SM/J and NZB/BINJ intercross that was mentioned previously (Stylianou et al. 2006). *Obwq4* maps near *D17Mit20* (Stylianou et al. 2006). Interestingly, a QTL for free fatty acid levels (regular chow) has been mapped to this region through analysis of intercross progeny derived from DBA/2 J and B6 (Colinayo et al. 2003). Although in our study the B6-derived allele decreased FFA levels relative to the A/J-derived allele, the B6-derived allele conferred higher levels relative to the DBA/2 J-derived allele (Colinayo et al. 2003). Candidate genes within the region include triglyceride interacting factor 1 (*Tgif1*), protein phosphatase 1B (*Ppm1b*), and lipin 2 (*Lipin2*). As with *Obrq13*, further fine-mapping studies are needed to narrow the region before candidate gene studies can be pursued.

***Obrq14* develops steatosis and insulin resistance**

Obrq14 maps to a region between markers m4 and m7. The A/J-derived allele promotes obesity within *Obrq14* and 17C5 becomes as obese as B6 even though the 17C5 congenic strain also contains the A/J-derived allele of *Obrq15*, which promotes resistance to obesity. In addition to promoting obesity, the A/J-derived allele of *Obrq14* also increases steatosis, fasting insulin, and cholesterol levels. The A/J-derived allele of *Obrq14* increases HOMA-IR to a level that is similar to B6, suggesting insulin resistance but further characterization of the degree and mechanism for insulin resistance is needed.

The *Obrq14* candidate region is much smaller (~ 15.9 Mb) than the candidate regions for the other two QTLs mapped using the congenic strains but still contains approximately 240 genes. Obesity-related QTLs previously mapped to this region include *Obwq4* described above (Stylianou et al. 2006). The *Obwq4* candidate region spans the candidate region for both *Obrq14* and *Obrq15* (Stylianou et al. 2006). Candidate genes within this QTL include NADH dehydrogenase (ubiquinone) 1 alpha sub-complex 11 (*Ndufa11*), and mitochondrial ribosomal proteins (*Mrpl14*, *Mrpl2*, *Mrps10*, *Mrps18a*). Mitochondrial dysfunction has been associated with type 2 diabetes and age-related insulin resistance (Kim et al. 2008). Oxidative stress, mitochondrial biogenesis, and genetic factors may contribute to overall mitochondrial function and ultimately lead to insulin resistance (Kim et al. 2008).

Congenic strains map more QTLs than intercrosses derived from CSS-17

Analysis of intercross progeny derived from CSS-17 and B6 detected a single QTL (*Obrq13*) near the centromere of chromosome 17 (data not shown). In contrast, the congenic strains provided evidence for at least three QTLs on the chromosome. The detection of multiple QTLs that influence obesity in congenic panels derived from CSSs has also been characterized for CSS-6 (Buchner et al. 2008) and CSS-10 (D. S. Sinasac and J. H. Nadeau, unpublished). Likewise, similar results have been obtained analyzing susceptibility to testicular cancer in congenic strains derived from a 129-Chr19^{Molf} CSS (Matin et al. 1999; Youngren et al. 2003). As in the present study, backcross studies using this CSS detected a single QTL for testicular cancer, whereas a congenic panel derived from 129-Chr19^{Molf} provided evidence for the existence of at least five QTLs (Matin et al. 1999; Youngren et al. 2003). Furthermore, genetic studies of the timing of puberty in CSS-6 demonstrated that a congenic panel spanning the chromosome allowed for finer mapping of a QTL relative to intercross studies (Nathan et al. 2006a, b). Thus, congenic strains are powerful for QTL detection.

Association among metabolic syndrome traits

Current animal models for steatosis focus on genetic mutations that promote increased lipogenesis or decreased fatty acid oxidation. Models for increased fatty acid synthesis include leptin deficiency (*ob/ob*), lack of leptin receptor (*db/db*), and altered leptin receptor signaling (*fa/fa*). Leptin, which is produced in adipose tissue, is a key appetite regulator in the brain. Lack of leptin or its receptor results in hyperphagia, which causes obesity, steatosis, and insulin resistance (Angulo 2002; Browning et al. 2004; Clark and Diehl 2003). Another animal model for steatosis has disrupted melanocortin receptor signaling in the hypothalamus. The melanocortin 4 receptor is an important regulator for appetite, body weight, and insulin secretion. Disruption of the melanocortin-4 receptor (MC4-RKO) causes obesity, steatosis, and insulin resistance (Butler and Cone 2001; Huszar et al. 1997). This has also been shown in *A^y* mice, which have a large deletion that places the agouti protein, a melanocortin-1 receptor (MC1-R) and MC4-R antagonist, under the control of a ubiquitously expressed promoter. The MC4-R expression is blocked and the yellow, obese phenotype results (Bultman et al. 1992; Lu et al. 1994; Michaud et al. 1993). In contrast, melanocortin 3-receptor (MC3-R)-deficient mice develop obesity but are protected from steatosis and severe insulin resistance (Ellacott et al. 2007). These mice have reduced inflammatory response to obesity, which has been suggested to be a potential mechanism for resistance to steatosis and insulin resistance (Ellacott et al. 2007).

Other genetic animal models have mutations that decrease lipid removal. The peroxisome proliferator receptor α -deficient mice (PPAR $\alpha^{-/-}$) have reduced fatty acid oxidation (Hashimoto et al. 2000). PPAR α is a hepatic transcription factor that regulates the transcription of mitochondrial and peroxisomal α -oxidation genes. Acyl CoA oxidase knockout mice (AOX $^{-/-}$) also have reduced fatty acid oxidation and develop severe hepatic steatosis and hepatomegaly (Fan et al. 1996). AOX is the first enzyme in the peroxisomal α -oxidation pathway.

These animal models were created using genetic manipulations that produce large effects on obesity and steatosis and may not represent the genetic variants found in NAFLD and NASH diseases in humans. Therefore, there is a need to identify better animal models for studying steatosis and the progression of liver injury in NAFLD/NASH. From this study we have identified three QTLs, *Obrq13* for which the A/J-derived allele protects against diet-induced obesity, steatosis, and elevated fasting glucose and insulin, *Obrq15* for which the A/J-derived allele protects against diet-induced obesity, and *Obrq14* for which the A/J-derived allele affects obesity, steatosis, and insulin resistance on a HFSC diet. Future studies will refine these regions and identify the genes and metabolic pathways that contribute to the resistance to NAFLD and may offer insight into potential therapies for NAFLD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Sequential analysis of CSS-17 congenic panel. Diagram of the tree used for sequential analysis of the metabolic traits studied with C57BL/6J as the root

Fig. 2. Growth curves and food intake on HFHS diet. CSS-17 (A17) and the congenic panel derived from CSS-17 were weighed weekly (**a**) and food intakes were measured weekly (**b**). The values are the mean $\pm$ SEM for $n = 8-22$ mice per group. * $p < 0.005$ compared with A/J; # $p < 0.005$ compared with B6; Bonferonni's correction for multiple testing. B6, C57BL/6J; A17, CSS-17; C1, 17C1; C2, 17C2; C3, 17C3; C4, 17C4; C5, 17C5; C6, 17C6; C7, 17C7

Fig. 3. Analysis of congenic strains on HFSC diet. Analysis of CSS-17 and the congenic strains derived from CSS-17 for the phenotype of (a) final body weight, (b) glucose, (c) insulin, (d) liver triglycerides, (e) cholesterol, (f) plasma triglycerides, and (g) plasma free fatty acids. A/J-derived segments are represented by shaded squares and the letter A. C57BL/6J-derived segments are represented by white squares and the letter B. Genetic markers are listed in order from centromere (left) to telomere (right) (m1 to m11) and are represented to scale showing their approximate location in the chromosome based on the location of markers obtained from the Mouse Genome Informatics website (<http://www.informatics.jax.org/>).

The number of mice tested in each group is in the column labeled Num. Mean phenotypes for each strain are shown by ovals with error bars representing SEM. Significant congenics are represented by the gray-filled ovals. Pairwise comparisons between strains are indicated, using two-tailed t tests with significance levels corrected for multiple hypothesis testing across the test performed. Chromosomal regions inferred to contain a QTL, based on a strong phenotypic difference between strains, are indicated

Fig. 4.

Analysis of congenic strains on LFSC diet. Analysis of CSS-17 and the congenic strains derived from CSS-17 for the phenotype of (a) final body weight, (b) liver triglycerides, and (c) plasma triglycerides. A/J-derived segments are represented by shaded squares and the letter A. C57BL/6J-derived segments are represented by white squares and the letter B. Genetic markers are listed in order from centromere (left) to telomere (right) (m1 to m11) and are represented to scale showing their approximate location in the chromosome based on the location of markers obtained from the Mouse Genome Informatics website (<http://www.informatics.jax.org/>). The number of mice tested in each group is in the column labeled Num. Mean phenotypes for each strain are shown by ovals with error bars representing SEM. Significant congenics are represented by the gray-filled ovals. Pairwise comparisons between strains are indicated, using two-tailed *t* tests with significance levels corrected for multiple hypothesis testing across the test performed. Chromosomal regions

inferred to contain a QTL, based on a strong phenotypic difference between strains, are indicated by shaded areas by shaded areas

Fig. 5. Glucose tolerance tests on HFSC diet. At the conclusion of the HFSC diet study (16 weeks), mice were fasted overnight. A 2-g/kg body weight intraperitoneal (IP) injection of glucose was given. Blood samples were taken from the tail vein at 0, 15, 30, 60, and 120 min after injection and glucose was measured by UltraTouch glucose meter. The values are the mean $\pm$ SEM for 6 mice per group; * $p < 0.005$

A

Strain comparison on HFSC diet	17C1 to B6	17C5 to 17C6	17C6 to 17C7
Contrasted QTLs	<i>Obrq</i> 13	<i>Obrq</i> 14 and <i>Obrq</i> 15	<i>Obrq</i> 15
Final BodyWeight (g)	↓	↑	↓
Glucose (mg/dL)	↓	≈	≈
Insulin (μg/l)	↓	↑	≈
Liver TG (mg/g liver)	↓	↑	≈
Plasma TG (mg/dL)	↑	↑	≈
FFA (mM)	≈	↓	↑
Cholesterol (mg/dL)	↓	↑	≈

B

Strain comparison on LFSC Diet	17C1 to B6	17C1 to 17C2	17C3 to 17C4	17C7to B6
Contrasted QTLs	<i>Obrq</i> 13	<i>Obrq</i> 17 and <i>Obrq</i> 13	<i>Obrq</i> 15 and <i>Obrq</i> 17	<i>Obrq</i> 16
Final Body Weight (g)	≈	≈	≈	≈
Liver TG (mg/g liver)	↓	≈	≈	↓
Plasma TG (mg/dL)	≈	↓	↑	≈

Fig. 6.

Congenic strains derived from CSS-17. Summary of the analysis of congenic strains derived from CSS-17 for the phenotypes of final body weight, glucose, insulin, liver triglycerides, plasma triglycerides, free fatty acids, and cholesterol after 16 weeks of HFSC diet. All congenic strains were compared with B6 mice. ↑ represents increased value, ↓ represents decreased value, and ≈ represents no effect