

ORIGINAL ARTICLE

Genetic control of obesity, glucose homeostasis, dyslipidemia and fatty liver in a mouse model of diet-induced metabolic syndrome

DS Sinasac^{1,5,6}, JD Riordan^{2,5}, SH Spiezio^{1,5}, BS Yandell³, CM Croniger^{4,7} and JH Nadeau^{1,2,7}

BACKGROUND/OBJECTIVES: Both genetic and dietary factors contribute to the metabolic syndrome (MetS) in humans and animal models. Characterizing their individual roles as well as relationships among these factors is critical for understanding MetS pathogenesis and developing effective therapies. By studying phenotypic responsiveness to high-risk versus control diet in two inbred mouse strains and their derivatives, we estimated the relative contributions of diet and genetic background to MetS, characterized strain-specific combinations of MetS conditions, and tested genetic and phenotypic complexity on a single substituted chromosome.

METHODS: Ten measures of metabolic health were assessed in susceptible C57BL/6 J and resistant A/J male mice fed either a control or a high-fat, high-sucrose (HFHS) diet, permitting estimates of the relative influences of strain, diet and strain–diet interactions for each trait. The same traits were measured in a panel of C57BL/6 J (B6)-Chr^{A/J} chromosome substitution strains (CSSs) fed the HFHS diet, followed by characterization of interstrain relationships, covariation among metabolic traits and quantitative trait loci (QTLs) on Chromosome 10.

RESULTS: We identified significant genetic contributions to nine of ten metabolic traits and significant dietary influence on eight. Significant strain–diet interaction effects were detected for four traits. Although a range of HFHS-induced phenotypes were observed among the CSSs, significant associations were detected among all traits but one. Strains were grouped into three clusters based on overall phenotype and specific CSSs were identified with distinct and reproducible trait combinations. Finally, several Chr10 regions were shown to control the severity of MetS conditions.

CONCLUSIONS: Generally strong genetic and dietary effects validate these CSSs as a multifactorial model of MetS. Although traits tended to segregate together, considerable phenotypic heterogeneity suggests that underlying genetic factors influence their co-occurrence and severity. Identification of multiple QTLs within and among strains highlights both the complexity of genetically regulated, diet-induced MetS and the ability of CSSs to prioritize candidate loci for mechanistic studies.

International Journal of Obesity (2016) 40, 346–355; doi:10.1038/ijo.2015.184

INTRODUCTION

The co-occurrence of multiple metabolic risk factors is known as the metabolic syndrome (MetS), the main characteristics of which include, but are not limited to, impaired glucose regulation and insulin resistance, abdominal obesity, dyslipidemia and hypertension.^{1–3} Although no universal definition of human MetS has been established, obesity is generally considered to be a key component and severities of additional conditions typically correlate with BMI.⁴ MetS substantially increases the risk of developing several pathological conditions, including cardiovascular disease, type 2 diabetes, stroke, certain forms of cancer and non-alcoholic fatty liver disease (NAFLD).^{2,3} Commonly referred to as the hepatic manifestation of MetS,⁵ NAFLD is characterized by lipid accumulation within hepatocytes and can progress to more severe conditions including non-alcoholic steatohepatitis, fibrosis, cirrhosis and liver cancer.⁶

Both genetic predisposition and environmental factors including dietary composition and physical activity contribute to the development of MetS.^{2,3} Its familial nature, variable prevalence among ethnic groups and increased concordance in monozygotic versus dizygotic twins all indicate a strong genetic component.^{7,8} More recently, human genome-wide association studies have identified several sequence variants associated with features of MetS,⁹ and animal models have directly demonstrated functional roles for a multitude of genetic and environmental factors.¹⁰ It is important not only to identify factors that contribute to MetS but also to understand how their mechanistic effects are influenced by context. For example, the relative phenotypic impact of a specific genetic variant generally depends on its interactions with environmental conditions and the broader genetic background.¹¹ At present, the largely unknown functional relationships among various etiological factors underlying MetS

¹Department of Genetics, Case Western Reserve University, School of Medicine, Cleveland, OH, USA; ²Pacific Northwest Diabetes Research Institute, Seattle, WA, USA;

³Department of Statistics, University of Wisconsin, Madison, WI, USA and ⁴Department of Nutrition, Case Western Reserve University, School of Medicine, Cleveland, OH, USA. Correspondence: Dr CM Croniger, Department of Nutrition, Case Western Reserve University, School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106, USA or Dr JH Nadeau, Pacific Northwest Diabetes Research Institute, 720 Broadway, Seattle, WA 98122, USA.

E-mail: colleen.croniger@case.edu or jnadeau@pnri.org

⁵These authors contributed equally to this work.

⁶These authors co-mentored the study.

⁷Current address: Alberta Children's Hospital Research Institute, Department of Medical Genetics, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada.

Received 18 May 2015; revised 24 July 2015; accepted 19 August 2015; accepted article preview online 18 September 2015; advance online publication, 13 October 2015

make it difficult to evaluate their relative importance, and more practically, to identify molecular targets for preventing, stabilizing or reversing disease.

Inbred mouse strains provide a powerful resource for studying complex traits like MetS. By minimizing the confounding effects of a genetically heterogeneous background, they facilitate functional investigations of the contributions of specific factors to phenotypes of interest. With careful study design, it is readily feasible to discover and characterize genetic, environmental and gene-environment interaction effects, identify disease genes and infer systems properties in simple and complex genetic conditions.^{12–15} Surveys of common inbred strains reveal a broad range of phenotypic diversity for traits related to diet-induced obesity and other metabolic conditions,^{16–18} implying that allelic variation strongly influences the outcome of dietary perturbations. Quantitative trait locus (QTL) mapping approaches, which identify associations between complex traits and the inheritance of specific allelic variants within defined genomic regions, can be utilized to enhance understanding of these genetic determinants of disease susceptibility.

Two commonly used inbred mouse strains, C57BL/6J (B6) and A/J, have contrasting responses to a high-fat, high-sucrose (HFHS) diet. B6 males develop obesity (predominantly mesenteric fat), hypertension, hypercholesterolemia, hyperinsulinemia and hyperglycemia,^{19,20} demonstrating high susceptibility to diet-induced MetS. By contrast, A/J males are resistant to these conditions, showing only modest phenotypic changes in response to the HFHS diet.^{19,20} Diet-induced liver phenotypes are also strikingly different. Whereas B6 males develop NAFLD characterized by progression to non-alcoholic steatohepatitis, fibrosis, cirrhosis and HCC, A/J males are resistant to these conditions.²¹

Early genetic studies of obesity and diabetes in B6 and A/J mice, using both traditional backcrosses and a panel of AXB/BXA recombinant inbred strains, provided preliminary evidence for causal genetic factors.^{22,23} Since then, a deeper appreciation for the complex nature of regulatory mechanisms and interactions influencing MetS susceptibility has emerged,^{24,25} indicating that more rigorous characterization of the model system may yield further important insights into MetS biology. A powerful approach to identify genetic loci influencing complex quantitative traits is the utilization of chromosome substitution strains (CSSs).²⁶ The B6-Chr^{A/J} CSS panel consists of 22 inbred strains (19 autosomes, both sex chromosomes and mitochondrial DNA), each containing a different non-recombinant homozygous A/J-derived chromosome substituted onto the B6 genetic background.²⁷ Unlike traditional crosses that utilize populations of genetically heterogeneous individuals to infer the presence, location and identity of a genetic variant, a CSS survey relies on phenotypic evaluation of multiple genetically identical individuals from each strain. An important distinction from other paradigms of complex trait analysis is that mean phenotypic differences observed between a CSS and the parental host strain (in this case B6) must be due to at least one allelic variant present on the substituted chromosome, without the complications of a heterogeneous genetic background.^{28,29} Focused study of CSSs and congenic strains derived from them has proven to be a particularly effective approach to identify causal genetic variants that regulate complex traits.³⁰

Our previous studies focused on gene identification, functional analyses, epistasis, transgenerational effects and systems properties in CSSs.^{27,31–33} In addition, detailed characterization of two substituted chromosomes revealed unexpected genetic complexity.^{34,35} In the present study, we focused on trait relationships. After estimating the relative influences of genetic and dietary factors and their interactions on susceptibility to 10 measures of diet-induced MetS, we examined patterns of covariation among metabolic traits across the complete B6-Chr^{A/J}

CSS panel and mapped QTLs on Chromosome 10 associated with each trait.

MATERIALS AND METHODS

Animal care

All animal procedures were approved by the Case Western Reserve University Institutional Animal Care and Use Committee. Mice were housed in one of two facilities – the Animal Resource Center or the Wolstein Animal Facility (WAF). For the CSS survey, both facilities were used for all strains except CSS-Mito, which was only analyzed in WAF. Because of the higher penetrance and severity of diet-induced MetS in males,^{25,27,36} no females were analyzed. For the intercross study, CSS-10 females were crossed to B6 males to produce F1 heterosomic progeny that were subsequently sibling mated. Resultant F2 progeny ($n = 120$ males) were analyzed in WAF. Construction of the CSS-A10 congenic panel has been described.³² For this study, all strains were analyzed in WAF. Standard mouse diet (Diet #5010; Purina LabDiet, St Louis, MO, USA) and autoclaved water were given *ad libitum* unless otherwise stated. At 5 weeks of age, randomly selected males were switched to HFHS diet (#D12331; Research Diets, New Brunswick, NJ, USA) for the remainder of each study (16 weeks). Sample sizes for each experimental group (listed in Tables 1 and 2) were determined based on statistical power calculations to provide a power of at least 0.80, α -level of 0.05.

Markers and genotyping

Microsatellite simple sequence length polymorphism and single-nucleotide polymorphism markers used for mapping experiments are listed in Supplementary Table 1, along with PCR primers and restriction endonucleases used for genotyping. All coordinates refer to the current mouse reference genome (GRCm38/mm10).

Plasma collection and metabolite assays

At 21 weeks of age, male mice were moved to a clean cage and fasted overnight. Each mouse was weighed and anesthetized via intraperitoneal injection with 0.8 mg g^{-1} of Avertin (2,2,2-tribromoethanol in tertiary-amyl alcohol; Fisher Scientific, Pittsburgh, PA, USA). Nose-to-anus length was measured to calculate BMI. Blood glucose was measured from the tail vein using a One Touch Ultra glucometer (Lifescan, Inc., Milpitas, CA, USA). Blood from the orbital sinus was drawn into a separation tube with gel barrier (StatSpin, Westwood, MA, USA) using a heparinized microhematocrit capillary tube (Fisher Scientific). Plasma was isolated and transferred to a new tube, immediately frozen on dry ice and stored at -80°C until analyzed. Plasma total cholesterol and triglycerides were measured with colorimetric reagents and standards according to the manufacturer's directions (Pointe Scientific, Canton, MI, USA). Plasma insulin was measured with a mouse ultrasensitive insulin ELISA kit according to the manufacturer's directions (Mercodia, Winston-Salem, NC, USA). Homeostasis model assessment of insulin resistance (HOMA-IR) assessment values were calculated as follows: $\text{HOMA-IR} = [\text{fasting insulin (mU l}^{-1}) \times \text{fasting blood glucose (mmol l}^{-1})] / 22.5$ as described previously.³⁷

Liver samples were frozen in liquid nitrogen and 100–200 mg of tissue was saponified in an equal volume by weight of $3 \text{ M KOH}/65\%$ ethanol to convert triglycerides to glycerol and fatty acids.³⁸ Glycerol was measured colorimetrically using a GPO-Trinder reagent set (Pointe Scientific) to quantify the triglyceride content of the original sample. Total liver triglycerides were estimated by multiplying the liver triglyceride content by total liver weight. No blinding strategy was used for these analyses.

Statistical analysis and genetic mapping

For all analyses, outliers were defined as individuals for which values for at least 1 of the 10 measured metabolic traits exceeded three times the interquartile range for that individual's strain; all measures obtained from outliers (51/879, 5.80% of mice) were excluded from further analysis. All reported P -values were adjusted for multiple hypothesis testing using Bonferroni correction.

Analysis of variance was performed on \log_2 -transformed values to identify significant effects of diet and strain, as well as their interactions, on measured metabolic traits. The effect of each factor was calculated after accounting for the others (i.e., type III sum of squares method) and adjusting for animal facility to account for any location effects. To identify trait associations in B6 and pooled CSS data sets, partial Pearson

Table 1. Effects of strain, diet and strain–diet interactions on MetS traits in C57BL/6 J and A/J parental strains

	Chow diet		HFHS diet		F-statistic (df ₁ = 1, df ₂ = 63)		
	C57BL/6 J (n = 17)	A/J (n = 15)	C57BL/6 J (n = 17)	A/J (n = 18)	Strain effect	Diet effect	Strain–diet interaction effect
Obesity							
Final body weight (g)	27.86 ± 0.49	24.09 ± 0.70	41.14 ± 1.40	29.82 ± 0.60	75.98***	125.90***	9.73 ^{NS}
BMI (g cm ⁻²)	0.26 ± 0.00	0.24 ± 0.01	0.38 ± 0.01	0.28 ± 0.00	77.82***	131.20***	18.02*
Glucose homeostasis							
Blood glucose (mmol l ⁻¹)	9.94 ± 0.39	9.08 ± 0.32	15.42 ± 1.04	11.82 ± 0.38	7.08 ^{NS}	32.91**	1.34 ^{NS}
Plasma insulin (pmol l ⁻¹)	60.67 ± 16.28	28.65 ± 3.50	295.84 ± 43.69	54.02 ± 6.37	16.48*	38.31**	9.48 ^{NS}
HOMA-IR	4.17 ± 1.22	1.63 ± 0.21	31.91 ± 5.65	3.99 ± 0.48	17.66*	44.93**	8.40 ^{NS}
Dyslipidemia							
Plasma total cholesterol (mmol l ⁻¹)	2.03 ± 0.08	1.56 ± 0.05	4.39 ± 0.22	2.96 ± 0.07	63.51**	308.51***	2.18 ^{NS}
Plasma triglycerides (mmol l ⁻¹)	0.57 ± 0.03	0.85 ± 0.11	0.49 ± 0.03	0.96 ± 0.06	36.73**	0.09 ^{NS}	5.06 ^{NS}
Hepatic steatosis							
Liver weight (g)	1.15 ± 0.04	0.91 ± 0.02	1.54 ± 0.09	0.86 ± 0.02	101.10***	7.11 ^{NS}	17.70*
Liver triglycerides (mg g ⁻¹ liver)	72.88 ± 5.28	62.93 ± 8.91	206.53 ± 14.09	79.00 ± 5.46	44.67**	56.72**	16.29*
Total liver triglycerides (mg)	82.51 ± 5.28	59.27 ± 9.22	332.24 ± 36.67	68.37 ± 5.43	80.78***	49.86**	22.65**

Abbreviations: ANOVA, analysis of variance; df, degrees of freedom; HOMA-IR, homeostasis model assessment of insulin resistance; NS, not significant. Trait values are listed as means ± s.e.m. Log₂-transformed values were used to calculate F-statistics for each factor (i.e., diet and strain) after accounting for the other using two-way ANOVA. P-values were adjusted by Bonferroni correction for multiple hypothesis testing. *P < 0.05; **P < 1.0 × 10⁻³; ***P < 1.0 × 10⁻¹⁰.

correlation analysis was performed on log₂-transformed mean values with adjustment for animal facility and strain using the ppcor package³⁹ in the R statistical software environment.⁴⁰

Unsupervised hierarchical clustering of strains based on log₂-transformed average metabolic trait values was performed using the R package pvclust⁴¹ with 10 000 bootstrap replications. Dendrograms were generated after adjusting for facility using average linkage clustering and uncentered correlation distance. Adjusted mean trait values normalized to B6 were used to generate a heat map with Java TreeView software.⁴² Confidence intervals for trait relationships to BMI were calculated based on a multivariate t-distribution using the stat_ellipse function in the R package ggplot2.⁴³

A total of 120 F2 males were phenotyped for the CSS-10 × B6 intercross, and interval mapping of QTLs was performed for each trait independently using the R/qtl software package.⁴⁴ Haley–Knott regression⁴⁵ was used to calculate LOD scores (log₁₀(1/p)), with permutation testing (n = 10 000) used to determine significance thresholds. LOD scores exceeding chromosome-wide significance thresholds of α = 5% and 63% were used to define significant and suggestive QTLs, respectively, as described previously.⁴⁶

RESULTS

Diet-induced metabolic traits in parental strains

We initially validated the ability of our experimental conditions to recapitulate the reported contrasting metabolic responses of C57BL/6 J and A/J mice to high-fat diet^{19–21} by comparing metabolic phenotypes in each strain after 16 weeks exposure to HFHS versus control diet. Ten traits related to obesity (final body weight and BMI), glucose homeostasis (fasting blood glucose, plasma insulin and HOMA-IR), dyslipidemia (plasma total cholesterol and triglycerides) and hepatic steatosis (liver weight, liver triglyceride concentration and total liver triglyceride content) were measured in each strain on HFHS (test) and standard chow (control) diets (Figure 1; Table 1). Trait values were similar for each strain on the control diet. In contrast, A/J mice remained lean and insulin sensitive, had lower plasma cholesterol and resisted steatosis on the HFHS diet compared with B6. Our results are consistent with previous studies implicating an underlying genetic component to these diet-induced metabolic conditions.^{19–21}

Genetic background, dietary composition and gene–diet interactions each influence MetS risk in humans.⁴⁷ The relative

contributions of these factors to phenotypic differences between our experimental cohorts were assessed with factorial analysis of variance (Table 1). Among the identified significant effects, strain had the largest influence on plasma triglycerides, liver weight and total liver triglycerides, whereas diet had the largest influence on plasma cholesterol and glucose homeostasis traits. Strain and diet had comparable individual effects on obesity traits and liver triglyceride concentration. Significant strain–diet interaction effects were detected for BMI and all three liver traits.

Genetic control of diet-induced obesity and metabolic traits in CSS panel

On the basis of our confirmation of a significant genetic component of differential susceptibility to diet-induced metabolic disease in the parental strains, we assessed the relative susceptibilities to various phenotypic aspects of disease in the complete B6-Chr^{A/J} CSS panel. The same 10 metabolic traits were measured in an average of 34 males for each of the 22 CSSs and both parental strains after HFHS diet for 16 weeks. We observed a range of phenotypes among strains with regard to the nature and severity of metabolic disease (Table 2), indicating that genetic contributions to diet-induced pathophysiology in this model are likely complex. Overall, 71 significant deviations from B6 traits were detected in the CSSs. In most (57/71) cases, phenotypes were less severe, as expected based on the lower disease susceptibility of A/J. Interestingly, although the parental strains did not differ for fasting glucose or plasma triglycerides, three CSSs differed from B6 mice for glucose levels and nine for plasma triglycerides (Table 2). It should be noted that mice in this study were housed in two distinct animal facilities, a fact accounted for by inclusion of facility as a covariate in the analyses.

Unsupervised hierarchical clustering based on log₂-transformed metabolic trait values was used to examine phenotypic similarities among CSSs, resulting in the identification of three seemingly distinct groups (Figure 2a). Deviation from B6 for each strain and trait was plotted as a paired heat map. The six CSSs that clustered with B6 displayed the most severe phenotypes, whereas the least severe phenotypes were observed in the four CSSs that clustered with A/J; the remaining 12 strains formed a cluster with intermediate phenotypes (Figures 2a and b).

Table 2. Mean trait values for measures of HFHS diet-induced obesity, glucose homeostasis, dyslipidemia and hepatic steatosis

Strain name	No. of mice (n)	Final body weight (g)	BMI (g cm ⁻²)	Blood glucose (mmol l ⁻¹)	Plasma insulin (pmol l ⁻¹)	HOMA-IR	Plasma total cholesterol (mmol l ⁻¹)	Plasma triglycerides (mmol l ⁻¹)	Liver weight (g)	Liver triglycerides (mg g ⁻¹ liver)	Total liver triglycerides (mg)
C57BL/6J	40	43.18 ± 0.98	0.38 ± 0.01	13.82 ± 0.58	312.35 ± 37.34	30.30 ± 4.11	4.04 ± 0.11	0.62 ± 0.05	1.52 ± 0.05	197.85 ± 12.29	322.70 ± 30.11
A/J	38	25.94 ± 0.42 ^a	0.25 ± 0.00 ^a	13.02 ± 0.20	45.98 ± 3.12 ^a	3.71 ± 0.24 ^a	2.80 ± 0.04 ^a	0.59 ± 0.04	0.85 ± 0.01 ^a	59.34 ± 2.42 ^a	50.19 ± 2.06 ^a
CSS-1	27	44.52 ± 0.70	0.40 ± 0.01	16.48 ± 0.48	316.51 ± 23.70	33.58 ± 2.87	4.85 ± 0.16	0.56 ± 0.03	1.57 ± 0.06	188.52 ± 11.04	309.83 ± 26.67
CSS-2	34	43.79 ± 1.01	0.41 ± 0.01	15.55 ± 0.36	531.58 ± 51.54	53.54 ± 5.57	4.33 ± 0.11	0.97 ± 0.04 ^b	1.48 ± 0.08	194.15 ± 12.89	321.01 ± 42.07
CSS-3	40	35.05 ± 0.75 ^a	0.32 ± 0.01 ^a	13.79 ± 0.63	137.79 ± 17.26	13.68 ± 2.07	3.57 ± 0.11	0.75 ± 0.04 ^b	1.19 ± 0.03 ^a	121.78 ± 9.17	153.11 ± 14.54 ^a
CSS-4	38	35.77 ± 0.62 ^a	0.32 ± 0.00 ^a	12.61 ± 0.39	178.69 ± 23.53	15.37 ± 2.25	3.22 ± 0.07 ^a	0.81 ± 0.04 ^b	1.14 ± 0.02 ^a	182.29 ± 7.51	211.99 ± 11.32
CSS-5	38	38.34 ± 0.60	0.35 ± 0.00	12.87 ± 0.40	154.83 ± 16.14	12.97 ± 1.46	4.01 ± 0.08	0.76 ± 0.04 ^b	1.16 ± 0.02 ^a	141.55 ± 8.96	168.76 ± 13.3 ^a
CSS-6	38	35.57 ± 0.61 ^a	0.32 ± 0.00 ^a	14.21 ± 0.34	244.84 ± 22.97	21.69 ± 1.88	3.72 ± 0.05	1.35 ± 0.06 ^b	1.18 ± 0.02 ^a	127.92 ± 6.30 ^a	154.37 ± 10.30 ^a
CSS-7	34	32.93 ± 0.69 ^a	0.31 ± 0.01 ^a	11.07 ± 0.56	73.99 ± 9.73 ^a	5.47 ± 0.86 ^a	3.87 ± 0.10	0.69 ± 0.04	1.07 ± 0.02 ^a	119.97 ± 7.99 ^a	130.15 ± 9.90 ^a
CSS-8	34	35.76 ± 0.65 ^a	0.32 ± 0.01 ^a	10.59 ± 0.44 ^b	101.44 ± 16.43 ^a	7.78 ± 1.48 ^a	3.45 ± 0.09 ^a	0.75 ± 0.05	1.08 ± 0.02 ^a	113.41 ± 3.17 ^a	122.57 ± 4.27 ^a
CSS-9	33	47.52 ± 0.82	0.41 ± 0.01	17.65 ± 0.44 ^b	351.55 ± 29.48	39.54 ± 3.64	4.35 ± 0.08	0.68 ± 0.04	1.83 ± 0.11	234.33 ± 16.22	481.33 ± 63.68
CSS-10	39	38.32 ± 0.92	0.34 ± 0.01	11.14 ± 0.37	98.16 ± 15.45 ^a	7.78 ± 1.42 ^a	3.40 ± 0.09	0.96 ± 0.04 ^b	1.12 ± 0.02 ^a	127.05 ± 3.45 ^a	143.55 ± 5.66 ^a
CSS-11	36	40.90 ± 1.12	0.37 ± 0.01	14.72 ± 0.52	250.10 ± 25.99	24.62 ± 2.87	4.08 ± 0.11	0.85 ± 0.04 ^b	1.31 ± 0.04	137.47 ± 9.86	192.63 ± 19.48
CSS-12	35	40.12 ± 0.77	0.36 ± 0.01	13.00 ± 0.65	169.21 ± 24.40	16.38 ± 2.90	3.75 ± 0.10	0.66 ± 0.03	1.32 ± 0.03	152.11 ± 7.24	207.26 ± 15.71
CSS-13	27	36.91 ± 0.73	0.34 ± 0.01	14.80 ± 0.62	300.10 ± 38.07	29.89 ± 4.08	3.80 ± 0.13	0.56 ± 0.03	1.24 ± 0.04	120.78 ± 9.49 ^a	157.40 ± 16.66 ^a
CSS-14	34	39.87 ± 0.81	0.36 ± 0.01	14.19 ± 0.45	231.05 ± 30.71	21.83 ± 3.31	4.19 ± 0.09	0.73 ± 0.03 ^b	1.18 ± 0.03 ^a	116.94 ± 4.99 ^a	140.25 ± 8.60 ^a
CSS-15	27	38.03 ± 0.98	0.34 ± 0.01	12.27 ± 0.58	146.20 ± 20.54	12.69 ± 2.19	3.37 ± 0.09	0.56 ± 0.03	1.20 ± 0.02 ^a	98.59 ± 4.80 ^a	118.38 ± 6.14 ^a
CSS-16	37	41.00 ± 0.84	0.36 ± 0.01	15.70 ± 0.50	321.25 ± 43.65	34.26 ± 5.26	4.08 ± 0.08	0.61 ± 0.02	1.25 ± 0.03 ^a	116.46 ± 4.69 ^a	148.44 ± 8.70 ^a
CSS-17	36	34.51 ± 0.67 ^a	0.31 ± 0.01 ^a	12.02 ± 0.46	62.70 ± 7.05 ^a	5.11 ± 0.69 ^a	3.89 ± 0.09	0.73 ± 0.04	1.11 ± 0.01 ^a	123.94 ± 3.37 ^a	137.77 ± 3.83 ^a
CSS-18	38	47.58 ± 0.94	0.43 ± 0.01 ^b	17.70 ± 0.54 ^b	359.81 ± 30.94	41.84 ± 4.18	4.65 ± 0.09 ^b	0.75 ± 0.04	1.70 ± 0.09	247.84 ± 13.94	463.66 ± 50.51
CSS-19	34	45.03 ± 0.98	0.41 ± 0.01	16.32 ± 0.68	413.97 ± 50.28	47.28 ± 6.77	4.61 ± 0.12 ^b	0.89 ± 0.06 ^b	1.44 ± 0.06	158.06 ± 9.15	241.73 ± 24.80
CSS-X	36	46.44 ± 0.73	0.41 ± 0.01	16.84 ± 0.34	522.14 ± 53.09	55.37 ± 5.69	4.54 ± 0.09 ^b	0.72 ± 0.04	1.66 ± 0.07	222.78 ± 13.00	395.97 ± 37.31
CSS-Y	31	38.39 ± 0.78	0.35 ± 0.01	14.86 ± 0.37	184.40 ± 19.23	18.01 ± 2.05	4.01 ± 0.10	0.67 ± 0.04	1.19 ± 0.03 ^a	137.52 ± 8.84	169.65 ± 15.70
CSS-Mito	24	37.56 ± 0.75	0.34 ± 0.01	14.97 ± 0.67	207.92 ± 28.77	21.36 ± 3.24	3.77 ± 0.11	0.65 ± 0.04	1.15 ± 0.02 ^a	123.71 ± 4.35	142.50 ± 6.60 ^a

Abbreviations: ANOVA, analysis of variance; CSS, chromosome substitution strain; HOMA-IR, homeostasis model assessment of insulin resistance. Trait values are listed as means ± s.e.m. Significant differences from C57BL/6J were identified using ANOVA on log₂-transformed values. ^aSignificantly lower than C57BL/6J (*P* < 0.05 after Bonferroni correction). ^bSignificantly higher than C57BL/6J (*P* < 0.05 after Bonferroni correction).

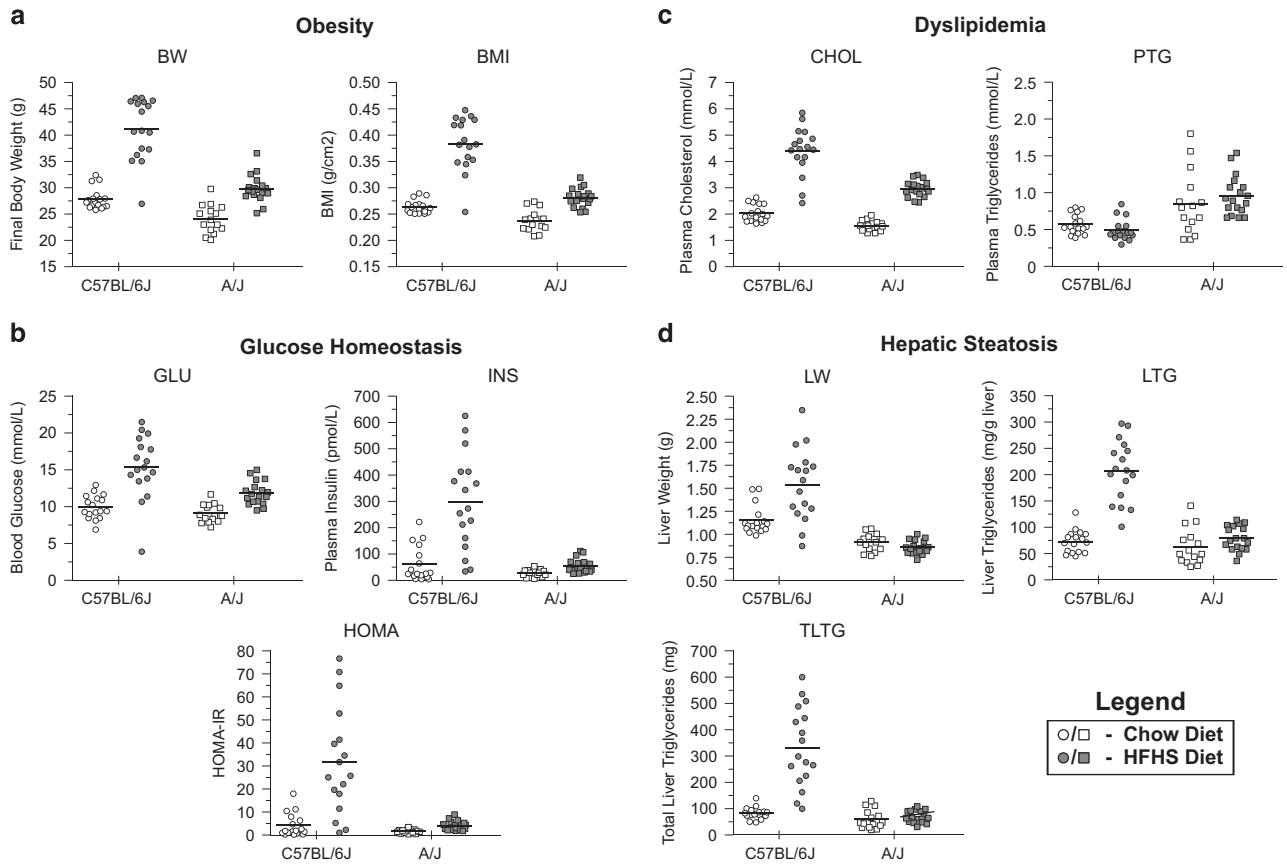


Figure 1. Metabolic responses of parental B6 and A/J strains to HFHS diet. Each dot represents the measured value for an individual mouse for 1 of 10 traits related to (a) obesity, (b) glucose homeostasis, (c) dyslipidemia or (d) hepatic steatosis after 16 weeks on either a normal chow (white symbols) or HFHS (grey symbols) diet. Horizontal bars indicate the mean value for each group. BMI, body mass index; BW, final body weight; CHOL, plasma cholesterol; GLU, blood glucose; HOMA, homeostatic model assessment; INS, plasma insulin; LTG, liver triglyceride content; LW, liver weight; PTG, plasma triglycerides; TLTG, total liver triglycerides.

Relationships among diet-induced metabolic traits in CSSs and B6. Scatter plots of measured trait values for each strain revealed clear positive correlations with BMI for blood glucose, plasma insulin, HOMA-IR, plasma cholesterol, liver weight and liver triglycerides, but not plasma triglycerides (Figure 2c). We also found that each cluster occupied a distinct, genetically determined physiological space within the overall pattern of variation among the CSSs. We next sought to determine either the B6 parental strain alone or the CSSs as a pooled population demonstrated additional relationships among MetS traits. For both B6 (Figure 3a, upper-right half) and the pooled CSSs (Figure 3a, lower-left half), significant positive correlations were found between all metabolic traits, with the exception of plasma triglycerides. Overall, trait relationships for the CSSs as a pooled population were highly similar to B6 (Figure 3b) and to those observed in humans,^{1–3} where pathological conditions strongly covary.

Interestingly, however, a subset of CSSs had reduced phenotypic severity for some traits but not others (Figure 3c). Each strain was categorized for the presence of obesity, insulin resistance, hypercholesterolemia and fatty liver based on statistical comparisons with B6 trait values. A condition was considered absent if values were significantly lower than B6 (Table 2). Nine CSSs shared all four conditions with B6. A/J and CSS-8 lacked all conditions, suggesting that A/J Chromosome 8 carries at least one QTL that confers full resistance to diet-induced MetS. Twelve CSSs had different combinations of conditions. CSS-7 and -17 were the only strains that showed a single B6-like condition (hypercholesterolemia). Among the other combinations, obesity never occurred without elevated cholesterol, and fatty liver never occurred

without insulin resistance. By contrast, insulin resistance and hypercholesterolemia did not depend on co-occurrence of any other condition. Through this analysis, we identified CSSs that are either fully resistant to MetS or to specific MetS conditions. These strains can be used to study the genetic and functional basis for resistance, as well as the molecular and physiological links between MetS conditions.

Mapping Chr10 QTLs associated with MetS trait susceptibility

We analyzed associations between inheritance of specific loci on Chr10 and trends in metabolic traits for both an intercross between B6 and CSS-10, where Chr10 was the only segregating chromosome, and a panel of CSS-10-derived congenic strains carrying distinct homozygous A/J-derived segments of Chr10. The intercross approach identified a single significant QTL peak for plasma cholesterol level ($LOD_{max} = 3.28$, 114 Mb), and several additional peaks passed the suggestive threshold (Figure 4): body weight ($LOD_{max} = 1.39$, 93 Mb), BMI ($LOD_{max} = 1.74$, 111 Mb), blood glucose ($LOD_{max} = 0.99$, 93 Mb), plasma insulin ($LOD_{max} = 2.13$, 83 Mb; $LOD_{max} = 1.79$, 129 Mb), HOMA-IR ($LOD_{max} = 1.69$, 83 Mb; $LOD_{max} = 1.49$, 129 Mb), plasma cholesterol ($LOD_{max} = 1.19$, 48 Mb), plasma triglyceride ($LOD_{max} = 2.15$, 49 Mb), liver weight ($LOD_{max} = 2.08$, 111 Mb) and total liver triglycerides ($LOD_{max} = 1.16$, 68 Mb; $LOD_{max} = 0.94$, 112 Mb). Given our observation of inter-relatedness among traits (Figure 3) and the dependence of some measures on others (e.g., HOMA-IR is calculated from glucose and insulin levels), overlapping peaks between QTL maps are expected and cannot

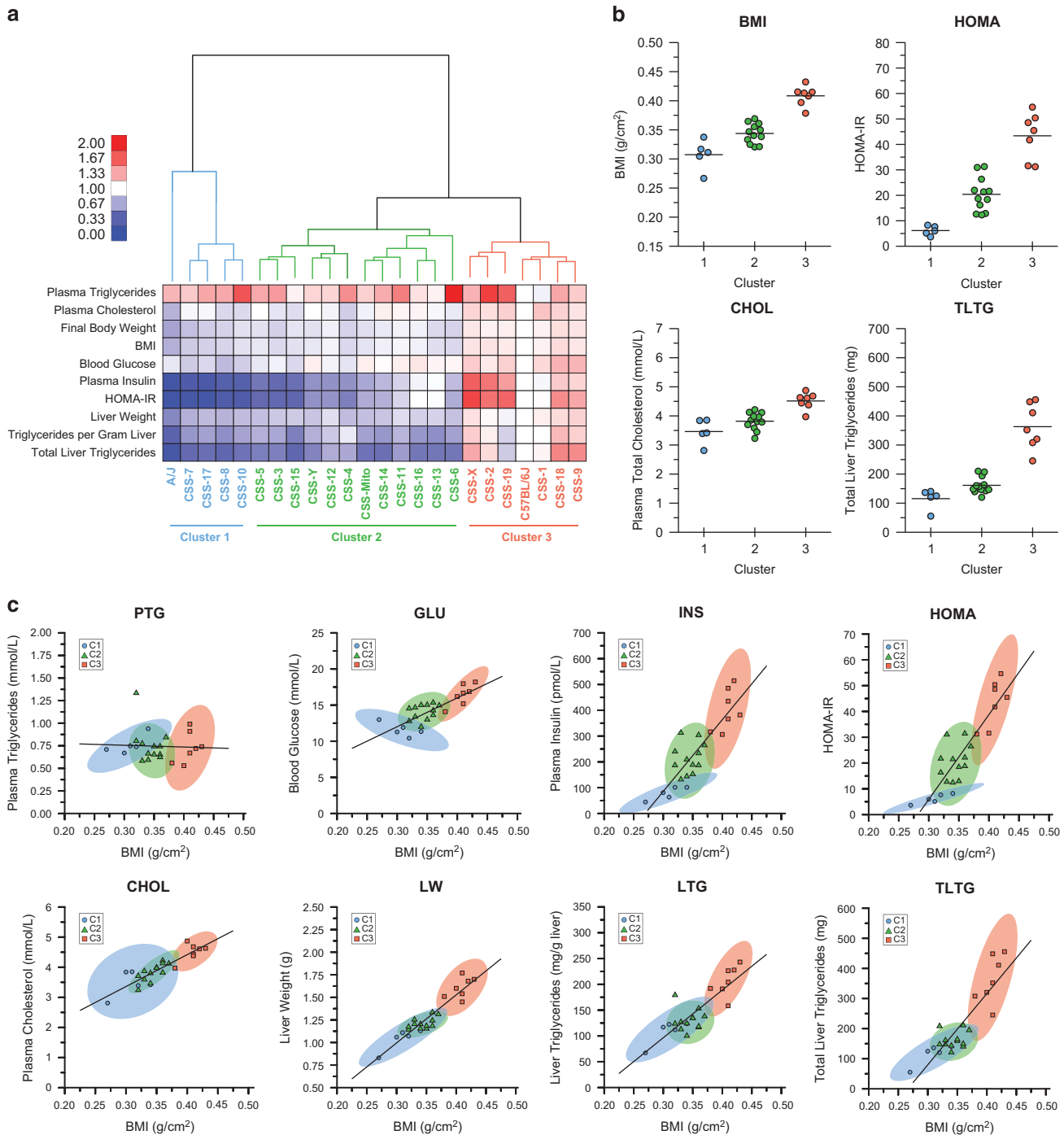


Figure 2. Metabolic characteristics of B6-Chr^{A/J} CSS panel on HFHS diet. **(a)** Interstrain relationships. Unsupervised hierarchical clustering based on metabolic traits identified three distinct strain groups. For the heat map, average trait values for each strain were normalized to those of B6, which were set to one. The color and degree of shading for each box indicate the direction and magnitude, respectively, of deviation from B6. **(b)** Diet-induced MetS severity by cluster. Each dot represents the average trait value for a single strain. Horizontal bars indicate the mean value among strains for each cluster. **(c)** Correlation of metabolic traits with BMI by cluster. Each dot represents the average trait value for a single strain. Shaded ellipses depict 95% confidence intervals for the xy coordinates indicative of each relationship in each cluster. Solid lines represent the best fit line for the data set as a whole.

be interpreted as evidence for a direct link between a particular Chr10 locus and multiple metabolic traits.

The detailed findings for a panel of 10 congenic strains derived from CSS-10 were published elsewhere,^{32,48} but are summarized here in the context of the present study. In brief, we located a total of 19 QTLs – four for diet-induced obesity (*Obrq9*, 126 Mb; *Obrq10*, 120 Mb; *Obrq11*, 90–93 Mb; *Obrq12*, 74–83 Mb), two for liver

triglycerides (*Ltgq1*, 58–68 Mb; *Ltgq2*, 120–126 Mb), four for blood glucose (*Gluq1*, 68–92 Mb; *Gluq2*, 92–104 Mb; *Gluq3*, 120–126 Mb; *Gluq4*, 126 Mb), two for plasma insulin (*Insq1*, 104–130 Mb; *Insq2*, 92–104 Mb), three for HOMA-IR (*Homaq1*, 126 Mb; *Homaq2*, 104–118 Mb; *Homaq3*, 82–103 Mb) and four for plasma cholesterol (*Pcholq1*, 126 Mb; *Pcholq2*, 102–126 Mb; *Pcholq3*, 82–103 Mb; *Pcholq4*, 67–89 Mb). The significant plasma cholesterol QTL

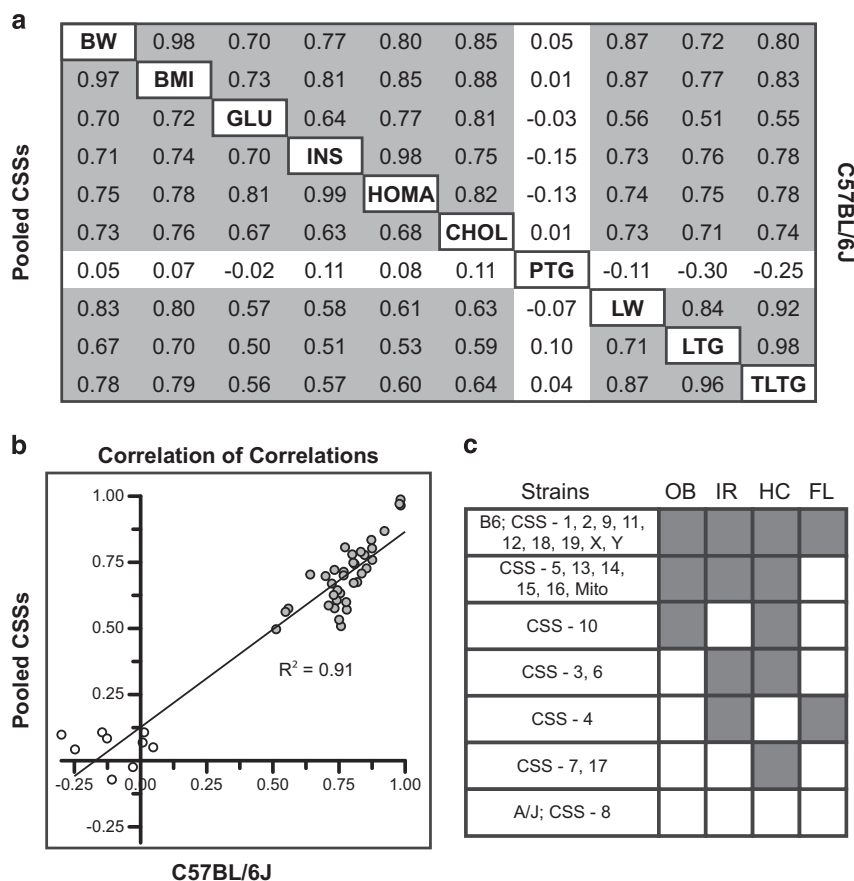


Figure 3. Correlations between measured metabolic traits in pooled CSS mice and in B6. **(a)** Partial Pearson correlation coefficients were calculated for all trait pairs based on \log_2 -transformed values adjusted for strain and facility. Results for the pooled CSS panel are shown on the lower left, while those for B6 are on the upper right. Correlation coefficients shaded in grey are statistically significant at $P < 0.05$ after Bonferroni correction for multiple hypothesis testing. **(b)** Comparison of pooled CSS and B6 trait correlations. For each trait pair in the scatter plot, the x and y values are the correlation coefficients (from panel A) for B6 and the pooled CSSs, respectively. Dot shading indicates significance of each correlation. **(c)** Strain categorization based on occurrence of individual MetS components. Obesity (OB), insulin resistance (IR), hypercholesterolemia (HC) and fatty liver (FL) were considered present in B6. For the remaining strains, these conditions were considered absent if significantly lower BMI, HOMA-IR, plasma cholesterol or total liver triglycerides were observed, respectively.

identified in our F2 cross (114 Mb) overlaps with the previously identified *Pcholq2* genetic variant.⁴⁸ In our experience, finding more genetic variants with CSSs and congenic surveys than with segregating crosses is not unusual.^{31,32}

Reproducibility of results

During the course of this work, we generated and characterized three independent cohorts for each parental strain (pilot study, CSS panel and mapping study) and two independent cohorts for the CSS-10 strain (CSS panel and mapping study). To assess the reproducibility of our results, we repeated the unsupervised hierarchical clustering analysis used to define the three distinct strain clusters (Figure 2a) with the inclusion of each repeated cohort as a separate entry. This analysis showed that independent cohorts of the same strain clustered tightly together in a dendrogram that closely resembles that of the original CSS panel (Supplementary Figure 1), indicating high reproducibility and thereby increasing confidence in our experimental results.

DISCUSSION

Understanding how genetics, diet and other environmental factors collectively contribute to the development of human metabolic disorders is an important but challenging task, especially given the need to deal with genetic and environmental

heterogeneity in a statistically robust manner.⁴⁹ Along with the established environmental influences of nutrition and physical activity,^{2,3} there is a growing appreciation for the involvement of other factors including social circumstances⁵⁰ and gut microbes,⁵¹ adding to the systems-level complexity. Despite these challenges, significant progress is being made. In 2007, the first robust evidence was provided of a link between human metabolic health and a common genetic variant.⁵² Since then, dozens of additional genes have been identified as regulators of human MetS-associated conditions.^{9,53} As with many other multifactorial conditions in humans, however, most MetS genetic variants have to date eluded discovery,⁵⁴ and the ways that genetic and environmental factors interact to modulate susceptibility remain poorly characterized.

A complementary approach to human studies takes advantage of spontaneous, induced, genetically engineered and naturally occurring variants in mouse models, where both genetic and environmental factors are more readily controlled.⁵⁵ Genetically engineered mouse models in particular have been valuable for characterizing molecular functions involved in diet-induced metabolic disease.^{56,57} However, the extent to which targeted activations or deficiencies recapitulate the heterogeneous conditions associated with MetS in humans is unclear. Dietary interventions with the inbred B6 and A/J mouse strains constitute an exceptional model for MetS because of their inherently divergent responses to high-fat diets, with B6 but not A/J showing

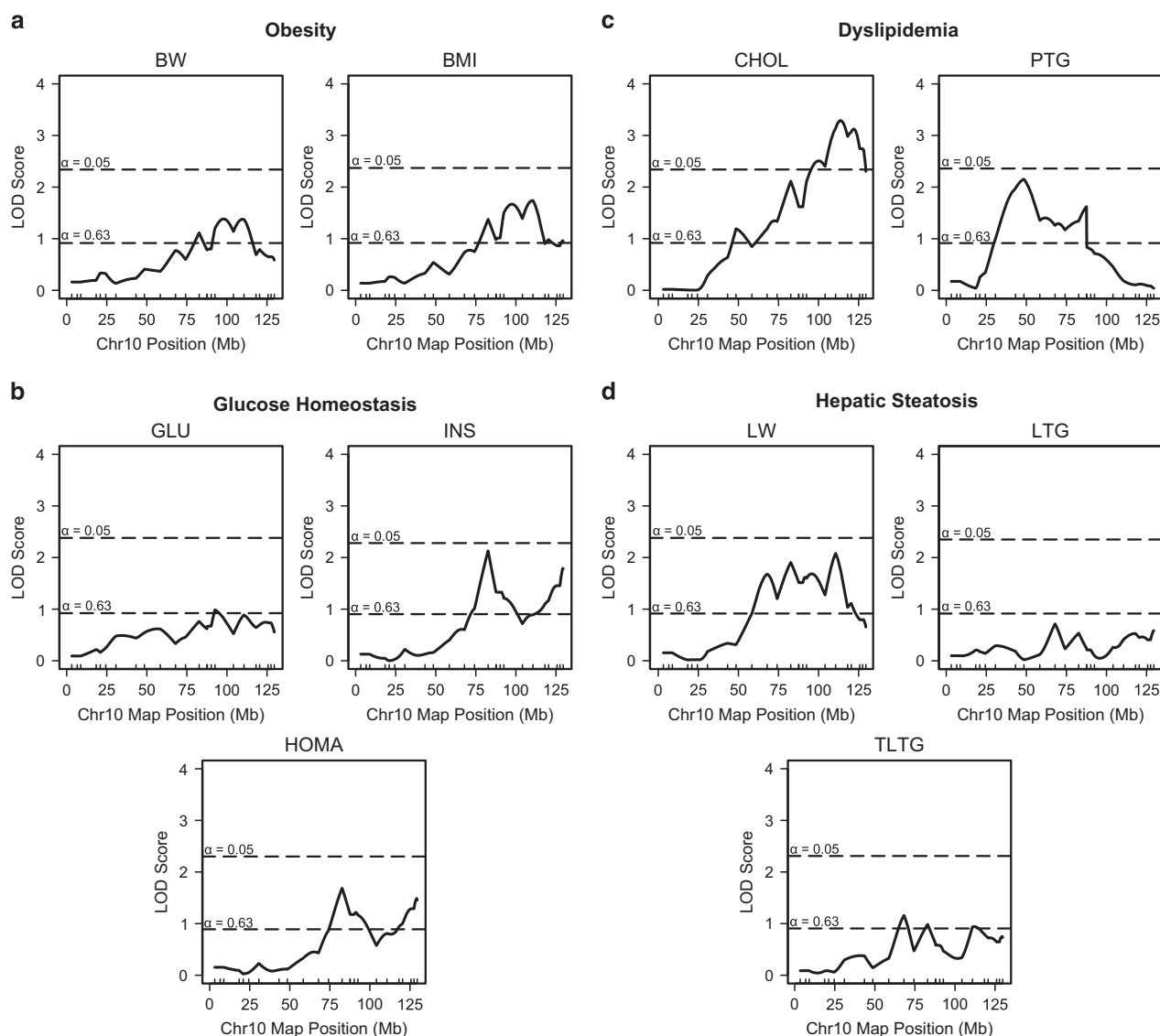


Figure 4. Mapping MetS QTLs in a (CSS-10 × B6) intercross. LOD scores (y axis) plotted along the length of Chromosome 10 for 10 traits related to (a) obesity, (b) glucose homeostasis, (c) dyslipidemia or (d) hepatic steatosis. Horizontal dotted lines indicate thresholds for significant ($\alpha = 0.05$) and suggestive ($\alpha = 0.63$) QTLs. Vertical ticks along the x axis show the positions of genotyping markers (detailed in Supplementary Table 1).

many features of MetS including insulin resistance, obesity, dyslipidemia and fatty liver disease (Figure 1, Table 1, see also Surwit *et al.*,²⁰ Hill-Baskin *et al.*²¹ and Surwit *et al.*²²).

In the present study, we examined several questions concerning diet-induced metabolic disease in the B6-A/J mouse model. We first assessed the relative contributions of strain (genetics), diet (environment) and their interactions on a set of MetS-associated traits (Table 1). It is interesting to note that while diet-induced responses differed significantly among strains (Table 1), a wide range of phenotypic severity was observed in susceptible B6 individuals (Figure 1). This result highlights the complex, multifactorial nature of metabolic physiology, even within a genetically homogeneous population under carefully controlled experimental conditions. We found that plasma triglyceride level was primarily influenced by strain. This result is consistent with recent large-scale human studies demonstrating high heritability of plasma triglycerides, as compared with other MetS components.^{58,59} In contrast, obesity (final body weight, BMI), plasma cholesterol, insulin resistance (fasting blood glucose, plasma insulin and HOMA-IR) and fatty liver disease (liver weight, liver triglycerides

and total liver triglycerides) were multifactorial, with both strain and diet contributing significantly to variation. Thus, as in human MetS,⁴⁷ various combinations of genetic and environmental effects, together with their interactions, influence the development of particular diet-induced conditions in this model.

In humans, many traits related to MetS show characteristic patterns of covariation, with obesity in particular representing a major predictor for several comorbidities.^{3,4} Similarly, B6 and the CSSs as a pooled population displayed extensive phenotypic covariation in this study, with strong positive correlations identified between all measured traits except plasma triglycerides (Figures 2c, 3a and b). These patterns may indicate the existence of physiological interdependencies among the covarying traits or a shared dependence on a separate, potentially unmeasured parameter. However, such functional interactions are challenging to demonstrate definitively in humans. While our study was not designed to establish causality, we were able to demonstrate highly significant phenotypic associations under conditions designed to minimize confounding effects. Along with the recapitulation of phenotypes and associations occurring in

humans, the strict control that can be achieved with the B6-Chr^{A/J} model makes it useful for defining and dissecting causal MetS relationships with clinical relevance.^{26,27} A notable advantage of the model is its ability to generate a range of phenotypes among individual strains and even uncouple distinct pathological aspects of MetS (Figure 3c). The present study identified considerable interstrain variability for fasting glucose and plasma triglycerides despite a lack of significant difference between parental strains (Table 2), implying that multiple genetic variants control the response of these traits to the HFHS diet or, in the case of plasma triglycerides, possibly independent of diet (Table 1). Findings such as these provide a unique opportunity to dissect complex genetic regulatory networks underlying the development of specific pathological traits and determine their interdependencies.

Replication has emerged as a major challenge in human genetic studies. Although more rigorous study designs are possible with animal models, variation among substrains and vagaries in environmental factors such as season, diet lot and bedding can lead to difficulties in reproducing the results among independent studies. We previously tested reproducibility among three CSS diet-induced obesity surveys in two different mouse facilities at Case Western Reserve University,³¹ finding that only 3 of 69 tests failed to replicate, and thus demonstrating that our methods are highly reproducible. Repeated cohorts in the present study also showed high reproducibility (Supplementary Figure 1). Tests for reproducibility are rarely undertaken with other mouse genetic reference populations, so assessing the relative merits of CSS surveys is not possible. Nevertheless, control of genetic background in CSSs together with rigorous husbandry conditions leads to strong reproducibility.

By comparing HFHS diet-associated metabolic traits across the B6-Chr^{A/J} CSS panel and parental strains, we identified 71 significant deviations from B6 conferred by substitution of a single chromosome (Table 2). While this information is useful as a starting point for deciphering the genetic basis of MetS, the ultimate goal is to map specific genes and polymorphisms that are important in determining susceptibility to MetS and its defining pathologies. CSS panels have considerable additional utility in this regard through the use of linkage crosses and congenic strains derived from individual CSSs.²⁶ To demonstrate this point in the context of the present study, we performed more detailed mapping studies with CSS-10. This strain was chosen because it clusters with A/J based on overall phenotype (Figure 2a), yet differs significantly from B6 for only insulin resistance and fatty liver (Figure 3c), a result that suggests the presence of multiple QTLs regulating distinct traits. We mapped 20 significant QTLs affecting MetS traits on Chr10, one in the CSS-10 × B6 intercross and 19 in the CSS-10 congenic strains (Figure 4, see also Shao *et al.*³² and Shao *et al.*⁴⁶). In addition, studies are required to definitively identify the gene(s) within these QTLs contributing to the observed phenotypes. However, candidates may be prioritized based on overlap with other mapping studies or human genome-wide association studies data. For example, the human ortholog of *Hmga2* (within the genomic regions defined by QTLs *Obrq10*, *Ltgq2*, *Gluq3*, *Insq1* and *Pcholq2*) has been associated with development of type 2 diabetes,⁶⁰ as has the *TSPAN8-LGR5* locus (mouse orthologs contained in *Insq1*, *Homaq2* and *Pcholq2*).⁶¹ On the basis of their connection to human metabolic dysfunction, these genes represent logical candidates for follow-up studies. We have also recently shown that subcongenic and subsubcongenic strains derived from CSSs can reveal many additional genetic variants with remarkably strong and non-additive phenotypic effects, a property we refer to as 'fractal genetics'.³³ Despite this extraordinary genetic complexity, we showed that underlying genetic variants could be readily identified. Studies like these demonstrate proof of concept that systematic characterization of precisely defined CSS disease models is a highly effective method

of identifying genetic variants with functional importance in various pathologies.

We provide here a comprehensive characterization of high-fat diet-associated metabolic health in the complete B6-Chr^{A/J} CSS panel. The precisely defined genetic composition of each inbred strain, along with their variable susceptibilities to conditions relevant to human MetS, provides a unique and powerful opportunity to decipher some of the genetic complexity underlying this ubiquitous condition. In addition to demonstrating the suitability of the B6-Chr^{A/J} mouse model for studying MetS traits relevant to human disease, our study provides a preliminary data set that will be useful for future targeted studies aimed at identifying genes involved in determining MetS susceptibility and severity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Mary Slaughter and Sudha Iyengar for helpful discussions. DSS was supported by a Postdoctoral Fellowship Award from the Canadian Diabetes Association. This work was supported by NIH grants RR12305 (JHN) and AA017837 (CMC).

REFERENCES

- Alberti KG, Zimmet P, Shaw J, IDF Epidemiology Task Force Consensus Group. The metabolic syndrome—a new worldwide definition. *Lancet* 2005; **366**: 1059–1062.
- Kaur J. A comprehensive review on metabolic syndrome. *Cardiol Res Pract* 2014; **2014**: 943162.
- Grundy SM, Brewer Jr HB, Cleeman Jr SC, Lenfant C, American Heart A *et al.* Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004; **109**: 433–438.
- Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. *JAMA* 1999; **282**: 1523–1529.
- Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M *et al.* Non-alcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; **50**: 1844–1850.
- Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 686–690.
- Poulsen P, Vaag A, Kyvik K, Beck-Nielsen H. Genetic versus environmental aetiology of the metabolic syndrome among male and female twins. *Diabetologia* 2001; **44**: 537–543.
- Tillin T, Forouhi NG. Metabolic syndrome and ethnicity. In: Byrne CD, Wild SH (eds). *The Metabolic Syndrome*, 2nd edn. Wiley-Blackwell: West Sussex, UK, 2011, pp 19–44.
- Fall T, Ingelsson E. Genome-wide association studies of obesity and metabolic syndrome. *Mol Cell Endocrinol* 2014; **382**: 740–757.
- Varga O, Harangi M, Olsson IA, Hansen AK. Contribution of animal models to the understanding of the metabolic syndrome: a systematic overview. *Obes Rev* 2010; **11**: 792–807.
- Civelek M, Lusis AJ. Systems genetics approaches to understand complex traits. *Nat Rev Genet* 2014; **15**: 34–48.
- Langfelder P, Castellani LW, Zhou Z, Paul E, Davis R, Schadt EE *et al.* A systems genetic analysis of high density lipoprotein metabolism and network preservation across mouse models. *Biochim Biophys Acta* 2012; **1821**: 435–447.
- Minkina O, Cheverud JM, Fawcett G, Semenkovich CF, Kenney-Hunt JP. Quantitative trait loci affecting liver fat content in mice. *G3 (Bethesda)* 2012; **2**: 1019–1025.
- Ghazalpour A, Rau CD, Farber CR, Bennett BJ, Orozco LD, van Nas A *et al.* Hybrid mouse diversity panel: a panel of inbred mouse strains suitable for analysis of complex genetic traits. *Mamm Genome* 2012; **23**: 680–692.
- Ghazalpour A, Bennett BJ, Shih D, Che N, Orozco L, Pan C *et al.* Genetic regulation of mouse liver metabolite levels. *Mol Syst Biol* 2014; **10**: 730.
- Svenson KL, Von Smith R, Magnani PA, Suetin HR, Paigen B, Naggert JK *et al.* Multiple trait measurements in 43 inbred mouse strains capture the phenotypic diversity characteristic of human populations. *J Appl Physiol (1985)* 2007; **102**: 2369–2378.
- Lin X, Yue P, Chen Z, Schonfeld G. Hepatic triglyceride contents are genetically determined in mice: results of a strain survey. *Am J Physiol Gastrointest Liver Physiol* 2005; **288**: G1179–G1189.

- 18 Parks BW, Nam E, Org E, Kostem E, Norheim F, Hui ST *et al*. Genetic control of obesity and gut microbiota composition in response to high-fat, high-sucrose diet in mice. *Cell Metab* 2013; **17**: 141–152.
- 19 Rebuffe-Scrive M, Surwit R, Feinglos M, Kuhn C, Rodin J. Regional fat distribution and metabolism in a new mouse model (C57BL/6 J) of non-insulin-dependent diabetes mellitus. *Metabolism* 1993; **42**: 1405–1409.
- 20 Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN. Diet-induced type II diabetes in C57BL/6 J mice. *Diabetes* 1988; **37**: 1163–1167.
- 21 Hill-Baskin AE, Markiewski MM, Buchner DA, Shao H, DeSantis D, Hsiao G *et al*. Diet-induced hepatocellular carcinoma in genetically predisposed mice. *Hum Mol Genet* 2009; **18**: 2975–2988.
- 22 Surwit RS, Seldin MF, Kuhn CM, Cochrane C, Feinglos MN. Control of expression of insulin resistance and hyperglycemia by different genetic factors in diabetic C57BL/6 J mice. *Diabetes* 1991; **40**: 82–87.
- 23 Seldin MF, Mott D, Bhat D, Petro A, Kuhn CM, Kingsmore SF *et al*. Glycogen synthase: a putative locus for diet-induced hyperglycemia. *J Clin Invest* 1994; **94**: 269–276.
- 24 Alevizos I, Misra J, Bullen J, Basso G, Kelleher J, Mantzoros C *et al*. Linking hepatic transcriptional changes to high-fat diet induced physiology for diabetes-prone and obese-resistant mice. *Cell Cycle* 2007; **6**: 1631–1638.
- 25 Hines IN, Hartwell HJ, Feng Y, Theve EJ, Hall GA, Hashway S *et al*. Insulin resistance and metabolic hepatocarcinogenesis with parent-of-origin effects in AxB mice. *Am J Pathol* 2011; **179**: 2855–2865.
- 26 Nadeau JH, Forejt J, Takada T, Shiroishi T. Chromosome substitution strains: gene discovery, functional analysis, and systems studies. *Mamm Genome* 2012; **23**: 693–705.
- 27 Singer JB, Hill AE, Burrage LC, Olszens KR, Song J, Justice M *et al*. Genetic dissection of complex traits with chromosome substitution strains of mice. *Science* 2004; **304**: 445–448.
- 28 Nadeau JH, Singer JB, Matin A, Lander ES. Analysing complex genetic traits with chromosome substitution strains. *Nat Genet* 2000; **24**: 221–225.
- 29 Belknap JK. Chromosome substitution strains: some quantitative considerations for genome scans and fine mapping. *Mamm Genome* 2003; **14**: 723–732.
- 30 Buchner DA, Nadeau JH. Contrasting genetic architectures in different mouse reference populations used for studying complex traits. *Genome Res* 2015; **25**: 775–791.
- 31 Burrage LC, Baskin-Hill AE, Sinasac DS, Singer JB, Croniger CM, Kirby A *et al*. Genetic resistance to diet-induced obesity in chromosome substitution strains of mice. *Mamm Genome* 2010; **21**: 115–129.
- 32 Shao H, Burrage LC, Sinasac DS, Hill AE, Ernest SR, O'Brien W *et al*. Genetic architecture of complex traits: large phenotypic effects and pervasive epistasis. *Proc Natl Acad Sci USA* 2008; **105**: 19910–19914.
- 33 Yazbek SN, Buchner DA, Geisinger JM, Burrage LC, Spiezio SH, Zentner GE *et al*. Deep congenic analysis identifies many strong, context-dependent QTLs, one of which, Slc35b4, regulates obesity and glucose homeostasis. *Genome Res* 2011; **21**: 1065–1073.
- 34 Buchner DA, Burrage LC, Hill AE, Yazbek SN, O'Brien WE, Croniger CM *et al*. Resistance to diet-induced obesity in mice with a single substituted chromosome. *Physiol Genomics* 2008; **35**: 116–122.
- 35 Millward CA, Burrage LC, Shao H, Sinasac DS, Kawasoe JH, Hill-Baskin AE *et al*. Genetic factors for resistance to diet-induced obesity and associated metabolic traits on mouse chromosome 17. *Mamm Genome* 2009; **20**: 71–82.
- 36 El Akoum S, Lamontagne V, Cloutier I, Tanguay JF. Nature of fatty acids in high fat diets differentially delineates obesity-linked metabolic syndrome components in male and female C57BL/6 J mice. *Diabetol Metab Syndr* 2011; **3**: 34.
- 37 Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004; **27**: 1487–1495.
- 38 Salmon DM, Flatt JP. Effect of dietary fat content on the incidence of obesity among ad libitum fed mice. *Int J Obes* 1985; **9**: 443–449.
- 39 Kim S-H. ppcor: partial and semi-partial (part) correlation. Available at: <http://cran.r-project.org/web/packages/ppcor/index.html> 2012.
- 40 Ihaka R, Gentleman R. R: a language for data analysis and graphics. *J Comput Graph Stat* 1996; **5**: 299–314.
- 41 Suzuki R, Shimodaira H. Pvcust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics* 2006; **22**: 1540–1542.
- 42 Saldanha AJ. Java Treeview—extensible visualization of microarray data. *Bioinformatics* 2004; **20**: 3246–3248.
- 43 Wickham H (ed). *ggplot2: Elegant Graphics for Data Analysis*, 8th edn. Springer: New York, NY, USA, 2009.
- 44 Broman KW, Wu H, Sen S, Churchill GA. R/qtl: QTL mapping in experimental crosses. *Bioinformatics* 2003; **19**: 889–890.
- 45 Haley CS, Knott SA. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* 1992; **69**: 315–324.
- 46 Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 1995; **11**: 241–247.
- 47 Roche HM, Phillips C, Gibney MJ. The metabolic syndrome: the crossroads of diet and genetics. *Proc Nutr Soc* 2005; **64**: 371–377.
- 48 Shao H, Sinasac DS, Burrage LC, Hodges CA, Supelak PJ, Palmert MR *et al*. Analyzing complex traits with congenic strains. *Mamm Genome* 2010; **21**: 276–286.
- 49 Newgard CB, Attie AD. Getting biological about the genetics of diabetes. *Nat Med* 2010; **16**: 388–391.
- 50 Campbell F, Conti G, Heckman JJ, Moon SH, Pinto R, Pungello E *et al*. Early childhood investments substantially boost adult health. *Science* 2014; **343**: 1478–1485.
- 51 Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012; **489**: 242–249.
- 52 Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM *et al*. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 2007; **316**: 889–894.
- 53 Anstee QM, Day CP. The genetics of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 645–655.
- 54 Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorf LA, Hunter DJ *et al*. Finding the missing heritability of complex diseases. *Nature* 2009; **461**: 747–753.
- 55 Lusis AJ, Attie AD, Reue K. Metabolic syndrome: from epidemiology to systems biology. *Nat Rev Genet* 2008; **9**: 819–830.
- 56 Butler AA, Cone RD. Knockout models resulting in the development of obesity. *Trends Genet* 2001; **17**: S50–S54.
- 57 Kennedy AJ, Ellacott KL, King VL, Hasty AH. Mouse models of the metabolic syndrome. *Dis Model Mech* 2010; **3**: 156–166.
- 58 Zarkesh M, Daneshpour MS, Faam B, Fallah MS, Hosseinzadeh N, Guity K *et al*. Heritability of the metabolic syndrome and its components in the Tehran Lipid and Glucose Study (TLGS). *Genet Res (Camb)* 2012; **94**: 331–337.
- 59 Zhang S, Liu X, Yu Y, Hong X, Christoffel KK, Wang B *et al*. Genetic and environmental contributions to phenotypic components of metabolic syndrome: a population-based twin study. *Obesity (Silver Spring)* 2009; **17**: 1581–1587.
- 60 Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP *et al*. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat Genet* 2010; **42**: 579–589.
- 61 Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T *et al*. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet* 2008; **40**: 638–645.

Supplementary Information accompanies this paper on International Journal of Obesity website (<http://www.nature.com/ijo>)