Calpain-10 is a component of the obesity-related quantitative trait locus *Adip1*

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Abstract **We previously mapped *Adip1*, an obesity quantitative trait locus (QTL), to the central portion of murine chromosome 1 containing the calpain-10 (*Capn10*) gene. Human studies have associated calpain-10 (*CAPN10*) variants with type 2 diabetes and various metabolic traits. We performed a quantitative hybrid complementation test (QHCT) to determine whether differences attributed to *Adip1* are the result of variant *Capn10* alleles in LG/J and SM/J mice. We crossed LG/J and SM/J to wild-type (C57BL/6J) and *Capn10* knockout ($Capn10^{-/-}$) mice to form four F_1 hybrid groups: LG/J by wild-type, LG/J by $Capn10^{-/-}$, SM/J by wild-type, and SM/J by $Capn10^{-/-}$. We performed a two-way ANOVA with the experimental strain, tester strain, and their interaction as the factors. Significant interaction indicates a quantitative failure to complement. We found failure to complement for fat, organ, and body weights, and leptin, female free fatty acid, and triglyceride levels. $Capn10^{-/-}$ resulted in heavier weights and higher serum levels in LG/J crosses but not in SM/J crosses. For glucose tolerance and insulin response tests, the $Capn10^{-/-}$ allele resulted in lower glucose levels in crosses with SM/J but had no effect in the LG/J crosses. Differences between the LG/J and SM/J *Capn10* alleles are the likely source of some of the QTL effects mapped to *Adip1* in the LG/J–by–SM/J cross. *Capn10* plays an important role in regulating obesity and diabetes in mice.—**Cheverud, J. M., G. L. Fawcett, J. P. Jarvis, E. A. Norgard, M. Pavlicev, L. S. Pletscher, K. S. Polonsky, H. Ye, G. I. Bell, and C. F. Semenkovich. **Calpain-10 is a component of the obesity-related quantitative trait locus Adip1. *J. Lipid Res.* 2010.** 51: **907–913.**

Supplementary key words diabetes • glucose tolerance • leptin • quantitative hybrid complementation test

We recently mapped *Adip1*, an obesity quantitative trait locus (QTL), to the middle portion of mouse chromosome 1, between microsatellite markers *D1Mit10* (92,584,545 bp) and *D1Mit139* (128,413,910 bp) in a set of LGXSM recombinant inbred (RI) strains (1, 2). This same region was found to affect body size at necropsy and body size growth between 3 and 10 weeks of age in replicate F_2 intercrosses of LG/J with SM/J mice (3, 4), the parent strains of the LGXSM RI strain set. In the RI line mapping study, this region was found to have significant effects on all four measured fat depots (reproductive, renal, mesenteric, and inguinal), body weight at necropsy, and serum leptin and cholesterol levels. These effects were observed in both sexes and on both high and low fat diets. The calpain-10 locus (*Capn10,* Chromosome 1 begins at 94,830,953 bp) is one of 134 genes identified in this QTL region (1).

The calpains are a family of cytoplasmic cysteine proteases (5, 6). Their physiological functions are poorly understood, but they have been implicated in the regulation of a variety of cellular processes including adipocyte differentiation (7, 8). One isoform, calpain-10, may also affect the risk of type 2 diabetes (9, 10). Calpain-10, which is ubiquitously expressed, may affect apoptosis in pancreatic islet cells (11), mitochondrial function (12), insulin secretion (13, 14), and oxidative utilization of glucose in muscles (15). Genetic variation in *CAPN10* has been associated with insulin resistance, dyslipidemia, and high fatty acid levels in a Japanese population (16); obesity in a Scandinavian population (17); and free fatty acid levels in a Finnish population (18). It has also been suggested that some *CAPN10* alleles confer higher risk for cardiovascular disease in those with diabetes (19). Human studies suggest that *CAPN10* variants may affect a large array of disease-

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Abbreviations: AUC, area under the curve; IPGTT, intraperitoneal glucose tolerance test; IRT, insulin response test; QHCT, quantitative hybrid complementation test; QTL, quantitative trait locus; RI, recombinant inbred, TG, triglyceride.

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related phenotypes, although replication of results across populations has proven difficult. The calpain-10 gene is also a very good candidate for being the locus responsible for *Adip1*, the obesity-related QTL.

Here, we test the hypothesis that *Capn10* is responsible for the observed QTL effects in populations derived from the LG/J–by–SM/J cross using a quantitative hybrid complementation test (QHCT) (20). This test has been successfully applied in QTL studies in *Drosophila* (21, 22), *Arabidopsis* (23), and mice (24, 25). This is not the same as an ordinary complementation test, but it has a similar interpretation. In a QHCT, two experimental strains (LG/J and SM/J) are each crossed with two tester strains, a mutant strain ($Capn10^{-/-}$) and its wild-type control (C57BL/6J). If the mutant–wild-type contrast is significantly different for crosses involving the two experimental strains, then there is a quantitative failure of the mutant to complement the experimental strain alleles. Quantitative failure to complement is indicated by a significant experimental-by-tester strain interaction among the four classes of hybrids (**Fig. 1**). A quantitative failure to complement signifies either that the QTL alleles at the locus of interest are responsible for the observed phenotypic variation or that other loci differing between the experimental strains interact with the mutant allele. In either case, the locus of interest is confirmed as being involved in trait variation. Here we cross experimental mouse strains LG/J and SM/J with a *Capn10* knock-out strain ($Capn10^{-/-}$) (H. Ye and G. I. Bell, unpublished observations) and its C57BL/6J wild-type control strain to test whether the *Capn10* gene may be responsible for the observed LG/J–by–SM/J intercross QTL effects.

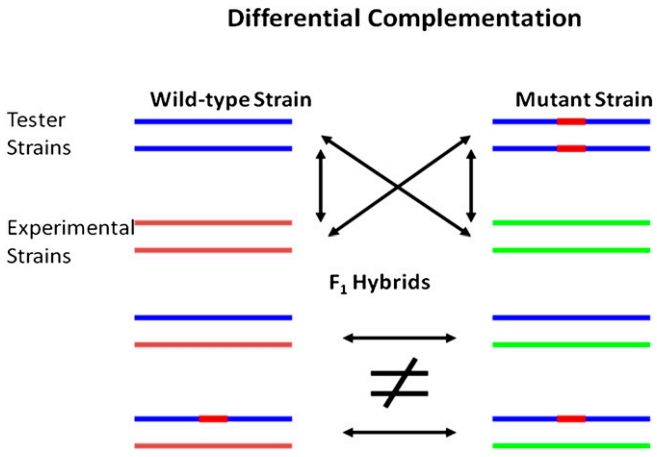

Fig. 1. Quantitative hybrid complementation test (QHCT) (20). Two experimental strains (LG/J and SM/J) are crossed to two tester strains (*Capn10* knockout and C57BL/6J wild-type) producing four sets of F_1 hybrids. When the difference between the mutant and wild-type hybrids changes, depending on the experimental strain they are mated to, there is a quantitative failure to complement as a result of differences between experimental strain alleles at the mutant locus or at other loci interacting with the mutant locus. The significance of failure to complement is given by the experimental-by-tester strain interaction.

MATERIALS AND METHODS

LG/J and SM/J are inbred strains selected for large or small body weight at 60 days (26) and have been used for a series of QTL mapping studies for body size, growth, obesity- and diabetes-related traits, and skeletal morphology (2, 27–29). Phenotypic differences between these two strains are typically caused by many genes of relatively small effect (1, 26). *Adip1*, one such QTL, is mapped to the central section of mouse chromosome 1, which contains the positional candidate gene *Capn10* (1).

The *Capn10* knockout mice were generated by replacing exons 1 and 2 with a selective cassette (nuclear-localized LacZ and neomycin-resistance genes) in R1 embryonic stem (ES) cells which were derived from a male blastocyst, hybrid of two 129 substrains (129 × 1/SvJ and 129S1/Sv-Oca2^{+}Tyr^{+}Kitl$^{Sl/J}$/J). Heterozygous *Capn10* knockout mice were backcrossed to C57BL/6J for six generations. The region of chromosome 1 between markers rs13475881 (nt 58,439,402) and rs6194543 (nt 157,588,922) is still 129 in origin.

LG/J and SM/J mice were obtained from Jackson Laboratories and bred to produce parents for the QHCT crosses. C57BL/6J wild-type mice were obtained from the Jackson Laboratories. LG/J and SM/J males were bred to *Capn10* knockout (mutant) and C57BL/6J (wild-type) females to produce four different sets of hybrid offspring: LG/J × *Capn10* knock-out (N = 116; 67♀:49♂); LG/J × C57BL/6J wild-type (N = 76; 38♀:38♂); SM/J × *Capn10* knock-out (N = 64; 33♀:31♂); and SM/J × C57BL/6J wild-type (N = 79; 41♀:38♂). Each animal's body weight was recorded weekly from 1 to 20 weeks. Pups were weaned at 3 weeks of age, transferred to single-sex cages of four or five animals, placed on a high-fat diet (Harlan Teklad #TD88137; **Table 1**), and maintained to 21 weeks of age. An intraperitoneal glucose tolerance test (IPGTT) was performed in week 19 by testing basal glucose level using a Glucometer Dex blood glucose meter (Bayer) after a 4-h fast, injecting 0.01 ml of a 10% glucose solution for each gram of body weight, and measuring subsequent glucose levels 15, 30, 60, and 120 min postinjection. From these measurements, the area under the curve (AUC) of glucose level plotted against time since injection was calculated for each animal. An insulin response test (IRT) was performed in week 20 by first measuring fasting (4-h fast) glucose level, then injecting 0.75 U of insulin per kg body weight into the peritoneum, and testing glucose levels at 15, 30, 60, and 120 min after injection. Animals experiencing insulin shock as denoted by severe ataxia, extreme lethargy, and glucose readings at or below 25 mg/dl were immediately injected with 0.25 cc of 10% glucose. All postinjection data for insulin shock animals were excluded from analysis. All procedures were consistent with the PHS Policy on Humane Care and Use of Laboratory Animals and approved by Washington University School of Medicine's Animal Studies Committee.

Animals were sacrificed using an overdose of sodium pentobarbital at 21 weeks of age after a 4-h fast, followed by blood collection via cardiac puncture. Serum levels of cholesterol, triglycerides (TG), free fatty acids, glucose, insulin, and leptin were assayed as described in Chakravarthy et al. (30). Body weight was recorded at necropsy and internal organs (heart, liver, kidneys, spleen) and fat depots (reproductive, renal, inguinal, mesenteric) were removed and weighed to the nearest 0.01 g.

A three-way ANOVA, including sex, experimental strain, tester strain, and their interactions as factors (31) was performed for each trait. Sets of traits were also analyzed jointly using MANOVA, including the fat depots, body and organ weights, time-specific glucose values for the IPGTT and IRT tests, and serum levels. When a significant three-way sex-by-experimental strain-by-tester strain interaction was detected, analyses of the strain types and their interaction were performed separately in males and females.

TABLE 1. Composition of High Fat Diet

Component	High Fat Diet
Calories from fat (%)	42
Casein (g/kg)	195
Sugars (g/kg)	341
Corn starch (g/kg)	150
Cellulose (g/kg)	50
Anhydrous milk fat (g/kg)	210
Cholesterol (g/kg)	1.5

Analyses were performed on both the raw and logarithmic scale, which produced similar results, so the raw scale is presented here for simplicity. Pairwise tests were also performed between the mutant and wild-type hybrids within the experimental strain for purposes of interpretation.

RESULTS

Means and standard deviations for all phenotypes in each of the hybrids, probabilities for differences between C57BL/6J wild-type and *Capn10* knockouts when crossed to either SM/J or LG/J, and the probability of no experimental-by-tester strain interaction are provided in **Table 2**. Analysis of the sum of the four fat pads indicates a significant experimental-by-tester strain interaction. Hybrids between the SM/J strain and the mutant and wild-type tester strains were not different in their levels of adiposity (**Fig. 2**). In contrast, the LG/J strain hybrids had much heavier fat depots when crossed with the *Capn10* knockout strain than with the wild-type C57BL/6J (2.32 g heavier, a 37% increase). Results are similar for the individual fat depots, with increases of 41%, 58%, 41%, and 19% for the reproductive, renal, mesenteric, and inguinal fat pads, respectively. Serum leptin showed a nearly significant interaction ($P = 0.075$) with the same pattern as that observed for the fat-pad weights. While a difference between mutant and wild-type was clear in the LG/J hybrids (50% difference; $P = 3.1 \times 10^{-5}$), the SM/J hybrids displayed a smaller (29%) but statistically significant ($P = 0.024$), difference between knockout and wild-type hybrids. Thus, leptin levels were higher in *Capn10* knock-out than wild-type hybrids, but the crosses with LG/J were not quite significantly more different from one another than the crosses with SM/J.

Body and internal organ weights, including the heart, kidneys, liver, and spleen, all show the same hybrid phenotypic pattern as the fat pads, with significant experimental-by-tester strain interactions. Percentage increase in body and organ weights between knockout and wild-type hybrids mated to LG/J ranged from a high of 34% for the liver to 18%, 16%, and 13% for body weight, heart weight, and spleen weight, with a low of 7% for the kidneys.

Serum free fatty acids ($P = 0.0084$) and triglycerides ($P = 0.0068$) showed significant three-way interactions, indicating different effects in males and females. Sex-specific analyses indicated significant interactions in females but not in males for FFA and TG. Patterns of hybrid differences for female FFA and TG are the same as noted above for fat pads, body, and organ weights. Serum cholesterol failed to show a significant experimental-by-tester strain interaction, although the *Capn10* knockout hybrid animals had consistently lower cholesterol levels than wild-type hybrids.

A three-way MANOVA with experimental strain, tester strain, and sex showed a significant three-way interaction indicating that the results of the QHCT differed between males and females for glucose response curves from the IPGTT (Probability = 3.1×10^{-6}). Therefore, we performed the test separately in males and females (**Fig. 3**). Males showed a very strong experimental-by-tester strain interaction. However, unlike the fat pads and organ weights, it is the SM/J hybrids, not the LG/J ones, that show the effect of the *Capn10* knockout. The difference between knockout and wild-type hybrids is not significant when they are mated to the LG/J strain. However, there is a large difference between knockout and wild-type hybrids when crossed to the SM/J strain. The SM/J–by–*Capn10* knockout males have much lower glucose levels (27% lower at the baseline, and 26% to 40% lower through the rest of the 2-h test) than the SM/J–by–C57BL/6J hybrid males. The experimental-by-tester strain interaction is borderline significant in females ($P = 0.051$), with significant interaction effects at 30 min and nearly significant effects at 60 min. The pattern of effects in females is similar to that in males but at a reduced effect size. As expected, the AUC results correspond to those given by the multivariate tests of IPGTT time-specific glucose levels, although here the females show a significant interaction effect ($P = 0.024$) (**Fig. 4**).

The insulin response test (IRT) results were similar to those reported for the IPGTT (**Fig. 5**), except that the three-way interaction was not quite significant at the 5% level (Probability = 0.073). Therefore, results provided are for the sexes pooled. The MANOVA identifies significant experimental-by-tester strain interaction, with the effect of the *Capn10* knockout being much stronger in the SM/J hybrids than in the LG/J hybrids. Neither serum insulin levels nor glucose levels at necropsy had significant experimental-by-tester strain interaction, although the *Capn10* knockout hybrids consistently had lower glucose levels than the wild-type C57BL/6J hybrids.

DISCUSSION

In our earlier QTL mapping study (1) utilizing the LGXSM recombinant inbred strains, we found a locus on chromosome 1 (*Adip1*), between genome coordinates 92,584,545 and 128,413,910 bp, with effects on all four measured fat depots (reproductive, renal, mesenteric, and inguinal); body weight at necropsy; serum leptin and cholesterol levels; and tail length. Our new findings confirm our earlier QTL results in that most of the traits affected by *Adip1* also failed to complement *Capn10*, including the four fat depots, leptin, and body weight at necropsy. In addition, organ weights showed a failure to complement, with strong interactions for liver and heart weights and weaker interactions for kidney and spleen weights. We also found interactions for serum free fatty acids and

TABLE 2. Mean, SE, and significance test for F_1 hybrids

Trait	SM-B6	SE	SM-C10	SE	Probability SM	LG-B6	SE	LG-C10	SE	Probability LG	Probability Int
Weight (g)											
Body	33.86	0.831	33.20	0.849	0.577	39.73	0.793	46.86	0.646	**8.0×10^{-11}**	**1.1×10^{-6}**
Tail	80.20	0.478	77.94	0.488	0.001	93.35	0.456	91.52	0.371	**0.002**	**0.639**
Heart	0.17	0.004	0.16	0.004	0.154	0.17	0.004	0.20	0.003	**4.7×10^{-7}**	**8.7×10^{-6}**
Kidney	0.19	0.004	0.19	0.004	0.567	0.20	0.004	0.21	0.003	**0.004**	**0.019**
Spleen	0.10	0.005	0.09	0.005	0.190	0.11	0.005	0.12	0.004	**0.021**	**0.012**
Liver	1.87	0.081	1.73	0.082	0.234	1.67	0.077	2.24	0.063	**6.7×10^{-8}**	**5.9×10^{-6}**
Fat depot (g)											
Reproductive	1.87	0.129	1.89	0.132	0.897	2.20	0.123	3.11	0.100	**4.1×10^{-8}**	**2.9×10^{-4}**
Renal	0.73	0.070	0.84	0.072	0.294	1.05	0.067	1.67	0.054	**3.7×10^{-11}**	**1.5×10^{-4}**
Mesenteric	0.78	0.053	0.84	0.054	0.390	1.01	0.051	1.42	0.041	**3.2×10^{-9}**	**6.5×10^{-4}**
Inguinal	1.64	0.102	1.49	0.104	0.306	2.02	0.097	2.41	0.079	**0.003**	**0.006**
Total fat	5.01	0.321	5.06	0.328	0.922	6.28	0.307	8.60	0.249	**2.4×10^{-8}**	**2.0×10^{-4}**
IPGTT ♀ (mg/dl)											
0 min	210	7.07	154	6.57	**4.6×10^{-8}**	185	7.47	181	6.16	0.626	**2.2×10^{-4}**
15 min	363	12.68	256	11.79	**3.5×10^{-7}**	306	13.41	304	11.06	0.933	**2.6×10^{-5}**
30 min	413	17.38	308	16.15	**1.4×10^{-4}**	309	18.37	332	15.15	0.275	**1.8×10^{-4}**
60 min	443	17.37	281	16.14	**3.7×10^{-8}**	293	18.36	309	15.14	0.433	**3.6×10^{-7}**
120 min	335	14.95	205	13.90	**4.8×10^{-7}**	203	15.81	230	13.04	0.101	**1.8×10^{-7}**
Multivariate					**2.1×10^{-8}**					0.320	**1.6×10^{-6}**
IPGTT ♀ (mg/dl)											
0 min	139	3.47	122	4.29	**0.009**	132	3.77	113	3.04	**2.9×10^{-5}**	0.783
15 min	233	6.39	203	7.92	**0.005**	193	6.94	179	5.60	0.097	0.242
30 min	228	6.36	185	7.88	**0.001**	174	6.91	172	5.57	0.763	**0.003**
60 min	176	5.43	145	6.72	**0.004**	153	5.89	144	4.75	0.189	0.057
120 min	129	4.35	113	5.39	0.084	111	4.72	107	3.81	0.332	0.230
Multivariate					**0.013**					**0.004**	0.051
IRT (mg/dl)											
0 min	162	3.45	146	3.82	**0.002**	146	3.50	145	3.16	0.909	**0.023**
15 min	142	3.81	107	4.21	**5.8×10^{-9}**	127	3.86	120	3.49	0.173	**3.5×10^{-4}**
30 min	111	5.27	88	5.83	**0.004**	111	5.34	95	4.83	**0.021**	0.548
60 min	107	3.97	93	4.39	**0.015**	96	4.03	96	3.64	0.983	0.074
120 min	136	3.72	115	4.12	**1.5×10^{-4}**	116	3.77	118	3.41	0.632	**0.002**
Multivariate					**9.4×10^{-7}**					0.153	**0.001**
AUC ♂ (minutes*mg/dl)	46338	1667	30711	1549	**1.4×10^{-10}**	32205	1762	34214	1453	0.380	**1.7×10^{-7}**
AUC ♀ (minutes*mg/dl)	21434	511	18068	632	**5.6×10^{-5}**	18006	555	17092	447	0.201	**0.025**
Serum level											
Leptin (ng/ml)	10.09	0.99	13.03	1.16	0.055	12.13	0.99	18.18	0.89	**1.1×10^{-5}**	0.124
Insulin (ng/ml)	1.60	0.14	1.56	0.16	0.848	1.62	0.14	1.90	0.12	0.127	0.247
Cholesterol (mg/dl)	173	5.17	135	6.05	**3.9×10^{-6}**	204	5.20	182	4.65	**0.002**	0.127
Glucose (mg/dl)	327	9.37	255	10.97	**1.4×10^{-6}**	330	9.42	267	8.43	**1.8×10^{-6}**	0.618
Free fatty acid ♀ (mmol/l)	1.92	0.23	1.48	0.25	0.197	3.23	0.23	1.70	0.18	**5.6×10^{-7}**	**0.016**
Free fatty acid ♂ (mmol/l)	1.99	0.12	1.19	0.13	**1.1×10^{-5}**	1.98	0.12	1.48	0.10	**0.002**	0.203
Triglyceride ♀ (mg/dl)	145	23	121	26	0.501	268	24	120	18	**2.6×10^{-6}**	**0.008**
Triglyceride ♂ (mg/dl)	161	10	106	11	**3.5×10^{-4}**	162	10	122	9	**0.003**	0.336

Hybrids are SM/J × C57BL/6J (SM-B6); SM/J × Capn10 knockout (SM-C10); LG/J × C57BL/6J (LG-B6); and LG/J × Capn10 knockout (LG-C10). Probabilities are given for differences in means between SM-B6 and SM-C10 (Probability SM) and LG-B6 and LG-C10 (Probability LG), and the probability of experimental-by-tester strain interaction (Probability Int), which is the test for a failure to complement. Significant probabilities are in boldface.

Abbreviations: AUC, area under the curve; IPGTT, intraperitoneal glucose tolerance test; IRT, insulin response test.

triglycerides in females. No interaction was discovered for cholesterol levels. Even so, *Capn10* hybrids had lower cholesterol levels than the C57BL/6J hybrids. Thus, there is strong congruence in phenotypes affected between the RI line QTL mapping and the QHCT experiments. Effects mapped to *Adip1* in the RI lines that do not show failure to complement in this study may be affected by other, nearby loci.

Our statistical findings clearly demonstrate that alleles from the SM/J and LG/J inbred mouse strains interact with the *Capn10* knockout mutation on a C57BL/6J background. This result is most likely due to differences between

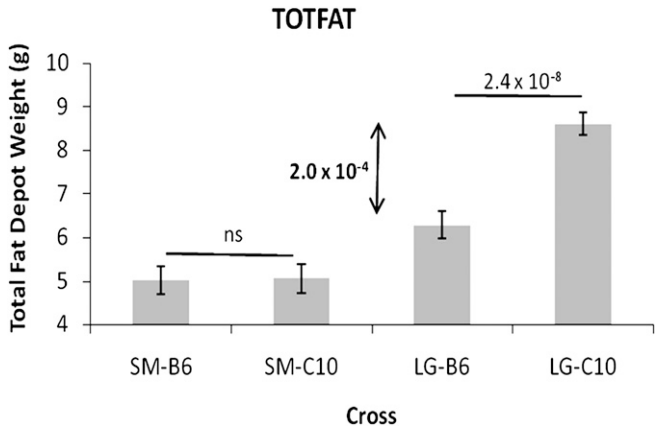

Fig. 2. Total fat depot weight for the four hybrid classes produced in the QHCT. SM, the SM/J strain; LG, the LG/J strain; C10, the *Capn10* knockout on a C57BL/6J strain background; B6, the wild-type C57BL/6J strain; NS, not significant at the 10% level. Probability of no interaction between experimental and tester strains appears to the left of the two-headed arrow. Probability of no difference between B6 and C10 hybrids crossed to either the SM/J or LG/J strain appears above the horizontal line. Error bars represent one standard error around the group mean. QHCT, quantitative hybrid complementation test. Error bars placed at the top of each column represent plus and minus one standard error around the group means.

the *Capn10* alleles in the LG/J and SM/J genomes. We predict that sequence differences in or near the *Capn10* locus are responsible for the observed quantitative failure to complement. These differences may be structural, as a result of coding differences between the LG/J and SM/J *Capn10* alleles, or because of expression differences in some or all the tissues expressing *Capn10*. It is also possible that the statistical interaction between LG/J and SM/J alleles and the *Capn10* knockout may not localize to the *Capn10* locus itself but be caused by epistatic interactions of *Capn10* with other loci segregating between LG/J and SM/J. We feel this is less likely than a *Capn10*-based difference between LG/J and SM/J because we previously mapped a QTL for the same traits that showed failure to complement to this region, and these QTL effects were not due to epistasis in the LGXSM RI strain study. However, further research is needed to eliminate the possibility of an epistatic source for the QHCT results. In either case, it is apparent that the *Capn10* locus plays an important role in modulating obesity and diabetes in these mice.

The pattern of failure to complement over all phenotypes was complex. For most obesity traits, including the fat depots, body weight at necropsy, organ weights, and serum leptin, free fatty acids, and triglycerides, hybrids involving the LG/J strain showed a difference between *Capn10* mutant and wild-type hybrids, whereas the mutant–wild-type difference was absent in hybrids formed with the SM/J strain. Hence the *Capn10* knockout leads to obesity, increased organ size, and increased serum leptin, free fatty acid, and triglyceride levels on a mixed C57BL/6J–by–LG/J hybrid background but not on a mixed C57BL/6J–by–SM/J hybrid background. Interestingly, this same combination of alleles and backgrounds has the opposite effect for the diabetes-related phenotypes, such as intraperitoneal glucose tolerance test (IPGTT) profile, area under the curve (AUC), and insulin response test (IRT) profile. For each of these phenotypes, the mutant–by–wild-type comparison is apparent in the crosses to SM/J but not in crosses to LG/J. Specifically, the wild-type–by–SM/J hybrids have a higher basal glucose level, higher AUC, and less complete recovery to baseline at 2 h than the *Capn10* knockout–by–SM/J hybrids. The *Capn10* knockout is protective against hyperglycemia when combined with the SM/J strain. The SM/J–by–C57BL/6J hybrids also respond poorly in the IRT, although this is primarily due to the higher fasting glucose levels in the SM/J–by–C57BL/6J hybrids. Again, the *Capn10* knock-out protects against diabetes. It is also of interest that the interaction effect, while present in both sexes, is much stronger in the males than females. Thus, the effects of the *Capn10* knockout are in opposite directions and occur in distinct backgrounds for obesity- and diabetes-related traits.

We also found sex-specific effects for levels of serum free fatty acid and triglyceride and for the IPGTT results. For the two serum components there is evidence that the effect of the *Capn10* knockout is only apparent in females from the LG/J crosses. The IPGTT effects have the same pattern and are statistically significant in both males and

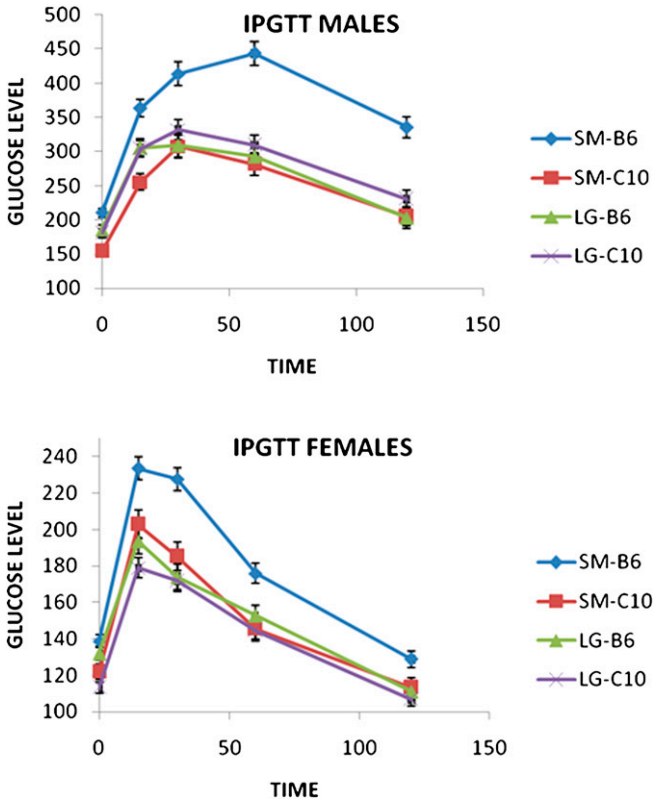

Fig. 3. Response to an IPGTT for males and females in the four hybrid groups in the QHCT. Mean glucose levels at each time point are plotted along with an error bar extending ±1 SE. The the *y* axis runs from 100 to 250 in females and from 100 to 500 in males. The glucose level scale (*y* axis) for males is twice that for females. IPGTT, intraperitoneal glucose tolerance test; QHCT, quantitative hybrid complementation test.

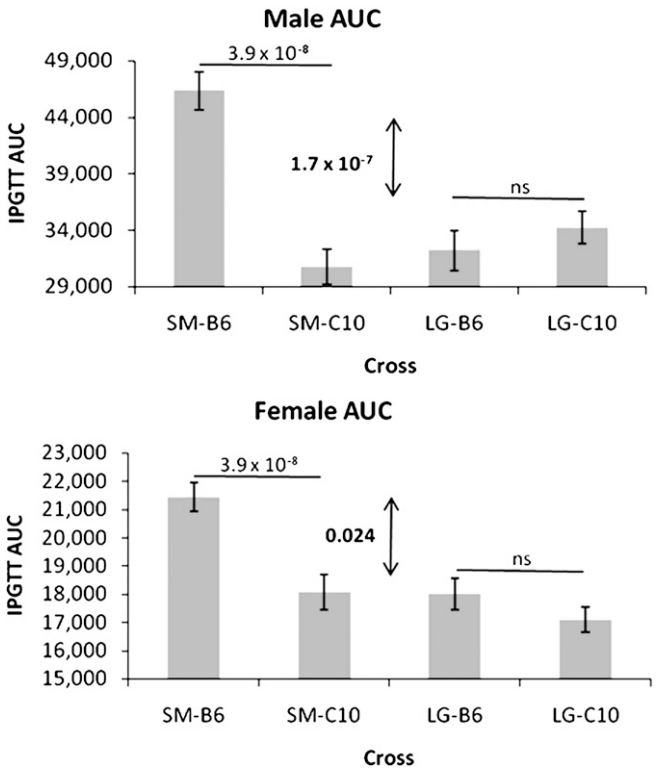

Fig. 4. AUC for males and females in the four hybrid groups in the QHCT. The AUC (*y* axis) scale for males is twice that for females. SM, the SM/J strain; LG, the LG/J strain; C10, the *Capn10* knockout on a C57BL/6J strain background; B6, the wild-type C57BL/6J strain. AUC, area under the curve; QHCT, quantitative hybrid complementation test.

females in the SM/J crosses but are much stronger in males than in females. These sex differences in the effects of *Capn10* are likely because of differences in the hormonal environment between the sexes and the consequences of these differences for *Capn10* expression and function. However, it is noteworthy that *Capn10* had similar effects in both sexes for all the other traits, including fat-pad weights, despite strong, ubiquitous sex-by-strain interactions reported for most of the traits analyzed here in

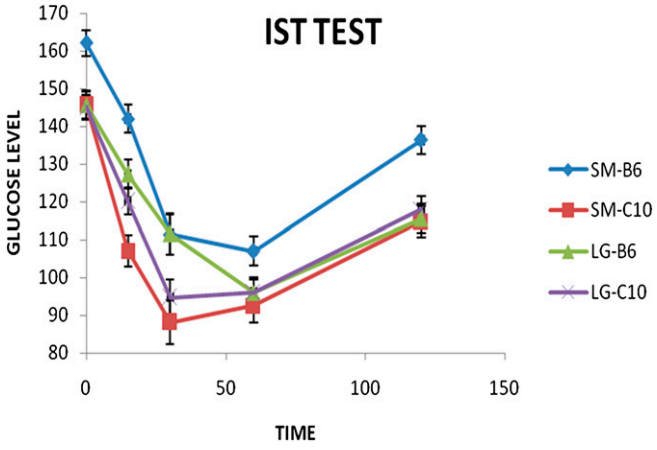

Fig. 5. Glucose levels for the duration of the IST for the four hybrid groups in the QHCT. IST, insulin response test; QHCT, quantitative hybrid complementation test.

the LGXSM recombinant inbred strains (32). Furthermore, *Adip1* did not display sex-specific effects (1). It appears that *Capn10* does not contribute much to the sex-specific effects noted in the LG/J–by–SM/J cross, although there is at least some genetic variation for sexual dimorphism in serum component levels and glucose response at the *Capn10* locus.

These differences in patterns of effects for obesity- and diabetes-related traits are consistent with our finding of a lack of correlation between obesity and diabetes phenotypes across the LGXSM RI strains (32). While it is possible that a single molecular change could lead to both an increase in obesity and a decrease in diabetes (or vice versa), we must also consider the possibility that there are multiple molecular differences between LG/J and SM/J at the *Capn10* locus with different molecular lesions leading to effects on different phenotypes. Other studies of *CAPN10* in humans suggest that different single nucleotide polymorphisms (SNPs) are often associated with different phenotypes (33). This may also be the case for the differential effects of LG/J and SM/J alleles.

In addition to the relevance of our findings with regard to genetic differences between LG/J and SM/J inbred mouse strains, our results confirm physiological effects of *Capn10* variants on a wide variety of obesity- and diabetes-related traits in mice. Obesity-related traits were strongly enhanced by the *Capn10* knockout in a LG/J–by–C57BL/6J hybrid background, whereas hyperglycemic diabetes-related traits were strongly reduced by the knockout in the SM/J–by–C57BL/6J hybrid background. In addition, the *Capn10* knockout resulted in lower necropsy serum glucose and cholesterol levels in both backgrounds. This range of effects complements previous findings regarding calpain-10's effects in various human populations. It also underscores the potential difficulty in replicating statistical and physiological effects in different human populations. The knockout allele clearly has different effects in different crosses as most findings in the LG/J–C57BL/6J contrasts are not replicated in the SM/J–C57BL/6J contrasts, and vice versa. Thus, most results are background-specific. A similar phenomenon in human populations has the potential to mask the effects of calpain-10 alleles in some populations for some traits but not for others.

CONCLUSION

We have found a quantitative failure to complement at the *Capn10* locus for obesity- and diabetes-related traits, supporting the hypothesis that this locus is, at least in part, responsible for *Adip1*, a QTL detected in earlier mapping studies of the LG/J–by–SM/J cross (1). It is likely that single or multiple molecular differences in or near the *Capn10* locus are responsible for obesity- and diabetes-related differences between the LG/J and SM/J inbred mouse strains. Our results confirm the effects of calpain-10 on a variety of obesity- and diabetes-related traits previously reported in human populations and support the hypothesis that failure to replicate for some traits in human populations may be due to variation in genetic background effects.

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