SGCG rs679482 Associates With Weight Loss Success in Response to an Intensively Supervised Outpatient Program

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Weight loss in response to energy restriction is highly variable, and identification of genetic contributors can provide insights into underlying biology. Leveraging 1000 Genomes imputed genotypes, we carried out genome-wide association study (GWAS) analysis in 551 unrelated obese subjects of European ancestry who participated in an intensively supervised weight loss program with replication of promising signals in an independent sample of 1,331 obese subjects who completed the program at a later date. By single nucleotide polymorphism–based and sib-pair analysis, we show that that weight loss is a heritable trait, with estimated heritability (h^2 = 0.49) within the range reported for obesity. We find rs679482, intronic to *SGCG* (sarcoglycan γ), highly expressed in skeletal muscle, to concordantly associate with weight loss in discovery and replication samples reaching GWAS significance in the combined meta-analysis (β = −0.35, $P = 1.7 \times 10^{-12}$). Located in a region of open chromatin, rs679482 is predicted to bind DMRT2, and allele-specific transcription factor binding analysis indicates preferential binding of DMRT2 to rs679482-A. Concordantly, rs679482-A impairs native repressor activity and increases basal and DMRT2-mediated enhancer activity. These findings confirm that weight loss is a heritable trait and provide evidence by which a novel variant in SGCG, rs679482, leads to impaired diet response.

Obesity is a complex phenotype (1), and energy intake, physical activity, non–exercise-associated energy expenditure, and genetic factors contribute to different degrees in a given individual. Weight loss variability in response to a variety of interventions is well-documented (2,3) and is in part a heritable trait (4). Bouchard et al. (5) demonstrated elegantly that identical twin pairs exhibit very high concordance for weight loss in response to exercise—in contrast to marked variability among dizygotic twin pairs. Hatoum et al. (6) reported that a 15q26.1 locus near *ST8SIA2* and *SLCO3A1* was significantly associated with weight loss after Roux-en-Y gastric bypass (RYGB). More recently, Heianza et al. (7) demonstrated that a common copy number variant in the amylase gene cluster confers greater weight loss in response to diet. In the Look AHEAD (Action for Health in Diabetes) trial, McCaffery et al. (8) used the Illumina CARe iSelect (IBC) chip to identify single nucleotide polymorphisms (SNPs) at the *ABCB11* and *TNFRSF11A* loci that associated with weight loss at year 1. Also in support of the hypothesis that weight loss post–bariatric surgery is not simply a function of dietary compliance, Hatoum et al. (9) found that first-degree relatives exhibit similar weight loss in response to RYGB compared with unrelated or, more interestingly, cohabitating individuals. Furthermore, we recently reported that weight loss in response to dietary intervention was highly correlated with weight loss following RYGB (10).

To investigate the biology underlying these differences, we have extensively characterized a large cohort of obese subjects enrolled in the Weight Management Clinic at The Ottawa Hospital. In support of the hypothesis that weight

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loss success has important molecular determinants, after screening for compliance and clinical conditions affecting weight loss, we reported that in comparison with diet-resistant subjects, diet-sensitive subjects exhibit increased proton leak in skeletal muscle (11), increased proportion of type I oxidative muscle fibers (12), and upregulation of OXPHOS genes in skeletal muscle (6) and in blood samples collected prior to the initiation of energy restriction (13).

Here, we have carried out a genome-wide association study (GWAS) for weight loss success in response to a controlled dietary intervention with replication in a separate sample. We also used these data to estimate the heritability of weight loss and to identify its risk factors through polygenic risk score (PRS) analysis. We provide further support for the hypothesis that this unique obesity phenotype is a heritable trait and identify a common polymorphism that disrupts a functional enhancer intronic to *SGCG* that associates with weight loss in response to energy restriction.

RESEARCH DESIGN AND METHODS

Study Subjects

Unrelated obese European white subjects (BMI 30–100 kg/m^2) (14) participated in an intensively supervised program at The Ottawa Hospital between 1998 and 2014. The intervention consisted of Optifast 900 meal replacement for 12 weeks followed by intensive diet and lifestyle counseling and weekly follow-up for 26 weeks. Patients were excluded from the study on the basis of medical conditions possibly affecting rate of weight loss, including thyroid indices (thyrotropin, free T3) out of normal range, diabetes treated with insulin or oral agents, cigarette smoking, congestive heart failure, obstructive sleep apnea, active malignancy, immobility, previous bariatric surgery, or treatment with weight-altering medications. Further analysis was limited to individuals who had adhered to ≥75% of the 26 weekly visits, had completed the laboratory testing protocol, and were deemed highly compliant by the treating physicians (14). Weight loss success was calculated as percent weight loss (PWL) on the basis of three repeat measures prior to and at the end of the 26-week program. Weight loss varied by twofold or more after correction for age, sex, and initial body weight. The study was approved by the Research Ethics Board of the University of Ottawa Heart Institute, and all participants provided written informed consent.

Genome-Wide Association Analysis

Discovery Sample

The discovery sample consisted of 551 unrelated subjects who participated in the weight loss program between 1998 and 2005, with available postimputed autosomal genotype data for 5,731,425 genome-wide SNPs.

Genotyping of the discovery cohort was carried out at the Ruddy Canadian Cardiovascular Genetics Centre using the Affymetrix Genome-Wide Human SNP 6.0 array (906,600 SNPs). SNPs with missingness >1%, Hardy-Weinberg equilibrium (HWE) *P* value <0.0001, or minor allele frequence (MAF) <0.01 and samples with >1% genotype missingness or discrepancies between the reported sex and sex determined from the X chromosome were excluded. The two main sources of genomic bias in GWAS studies are cryptic relatedness and population stratification. Identity-by-state (IBS) pruning was performed by excluding one of each pair of individuals with an estimated genome-wide IBS >0.05 and retained a subset of 551 unrelated individuals. The reason for excluding related individuals is avoidance of the possibility that the phenotypic resemblance between close relatives could be due to nongenetic effects (e.g., shared environment) and causal variants not tagged by SNPs but captured by pedigree. We carried out a genotype quality control procedure using the PLINK program (version 1.9) (15).

Genotypes were prephased using the SHAPEIT program (v1.ESHG) (16) followed by genotype imputation using IMPUTE2 (v2.2.2) (17) and based on the 1000 Genomes reference panel (phase 1, release 3). Following genotype imputation, we excluded SNPs with MAF <1%, missing values in >1% of individuals, HWE *P* value <0.0001, and imputation INFO score ≤0.9 prior to our GWAS. To correct for population structure, we conducted principal component analysis of ancestry, mapped subjects with samples from 1000 Genomes, and excluded those of non-European ancestry. We then used the first 10 computed principal components to further control for subtle population structure. GWAS analysis was carried out using SNPTEST (v2.5.2) (18). Prior to the GWAS analysis, we adjusted the weight loss phenotype for age, sex, and population stratification using linear regression model implemented in R (version 3.6.0).

Exploratory Sample

We tested the association of significant signals in another sample of 710 unrelated subjects of European ancestry who underwent the same weight loss program between 2005 and 2012, which was previously used to study the genetics of circulating miRNAs (19). In summary, subjects in this sample were genotyped using Illumina HumanCoreExome-12 v1.1, with 264,909 tag SNP markers and 244,593 exome-focused markers. We excluded SNPs with missingness >1%, HWE *P* value <0.0001, MAF <0.01, or discrepancies between the reported sex and sex determined from the X chromosome. Genotypes were then prephased using the SHAPEIT program (v1.ESHG) (16) and followed by genotype imputation using IMPUTE2 (v2.2.2) (17) and based on the 1000 Genomes reference panel (phase 1, release 3). Following genotype imputation, we excluded SNPs with MAF <1%, missing values in >1% of individuals, HWE *P* value <0.0001, and imputation INFO ≤0.9 and finished with postimputed genotype data for 4,831,554 genome-wide SNPs.

Replication Sample

The replication sample consisted of the exploratory sample and an additional 621 subjects who completed exactly the same 26-week dietary intervention protocol between 2000 and 2014. Unlike the discovery and exploratory samples, rs679482 was genotyped directly (without imputation) in

the replication sample using a TaqMan SNP assay kit (Thermo Fisher Scientific). This assay was performed on a Roche LightCycler 480 on a 384-well plate. The genotype score and call of the samples were determined using the end point analysis tool within the LightCycler 480 software v1.52 (Roche Diagnostics). Samples that scored <0.95 were repeated and rejected if the subsequent score was <0.95. Overall, 92% of samples passed quality control and were used for analysis.

Heritability Analysis

We used the genomic-relatedness-matrix restricted maximum likelihood (GREML) algorithm implemented in GCTA (Genome-wide Complex Trait Analysis) software (20) to estimate the narrow-sense heritability (h_g^2) of weight loss explained by all genome-wide SNPs. Previously we used this approach to estimate the heritability and coheritability of coronary artery disease and BMI (21,22). The GREML algorithm underperforms with small sample size; as such, we did this analysis in the combined sample made of discovery and the exploratory samples. For this purpose, we merged the postimputed genotype data from the two samples. We then excluded SNPs with MAF <1%, missing values in >1% of individuals, or HWE *P* value <0.0001 and generated a genomic relatedness matrix (GRM) from the genotype data in GCTA (v1.92.2) (12). We further excluded one of each pair of individuals with an estimated genetic relatedness >0.05. This provided a subset of 1,080 unrelated individuals with available 26-week weight loss and genotype data at 4,681,082 SNPs (12).

Separately, we determined interindividual rate of weight loss in 20 sex-matched sib-pairs having completed the program with 112 age- and sex-matched unrelated individuals.

PRS Analysis

Previously we carried out a phenome-wide search for traits that are causally associated with the risk of cardiovascular disease (23). Here, we tested whether these traits are also associated with weight loss success. For this purpose, we calculated individual weighted PRS for each trait as follows:

$$S = \frac{\sum_{i=1}^{i=M} x_i \beta_i}{M} \quad (1)$$

where M is the total number of independent SNPs that are nominally associated ($P < 0.01$) with a trait and also are genotyped in the combined sample, x_i is the count of effect alleles for the ith SNP in an individual from our sample with $x_i \in$ ("0_1,_2") and β_i, and β_i is the effect size of ith SNP, which we obtained from GWAS summary statistics. S is the polygenic score for an individual, and PRS is a vector of S values for all individuals in our sample (N = 1,080).

To obtain a list of independent SNPs that are associated with a trait, we used the clumping algorithm implemented in PLINK 1.9. In summary, this algorithm forms clumps around nominally associated SNPs ($P < 0.01$). Each clump contains all SNPs within 250 kb of the index SNP that are also in linkage disequilibrium with the index SNP ($r^2 \geq 0.5$). The algorithm iteratively cycles through all index SNPs, beginning with the smallest *P* value in a region, only allowing each SNP to appear in one clump. The final output contains the list of most significantly associated SNPs (index SNPs) for each clump across the genome.

We then tested the association between the computed PRS for a trait (PRS_{trait}) and coronary artery disease by fitting the following linear model for our study participants:

$$\text{Weight loss} \sim \text{sex} + \text{age} + \text{PCs} + PRS_{trait} \quad (2)$$

where PCs is first two principal components from our principal component analysis.

Meta-analysis

The β-coefficient estimates (β_i) from the discovery and the replication samples were pooled, and the weighted mean of β-coefficients ($\overline{\beta}$) was calculated based on the fixed-effect model (24) as follows:

$$\overline{\beta} = \frac{\sum_{i=1}^{n} w_i \times \beta_i}{\sum_{i=1}^{n} w_i} \quad (3)$$

where w_i is the inverse variance of the ith study and n is the number of studies.

SE of combined effect was calculated as follows:

$$SE(\overline{\beta}) = \sqrt{\frac{1}{\sum_{i=1}^{n} w_i}} \quad (4)$$

The two-tailed *P* value was calculated as follows:

$$P = 2 \times \left[1 - \Phi\left(\left|\frac{\overline{\beta}}{SE(\overline{\beta})}\right|\right)\right] \quad (5)$$

where Φ is the standard normal cumulative distribution function.

Cochran *Q* statistic, which measures the heterogeneity between studies, was calculated as follows:

$$Q = \sum_{i=1}^{n} w_i \times (\beta_i - \overline{\beta})^2 \quad (6)$$

Q has χ^2 distribution with $n - 1$ degrees of freedom.

I^2 index is another heterogeneity metric, which is expressed as percentage of the total variability in a set of effect sizes due to true heterogeneity. We calculated I^2 as follows:

$$I^2 = \frac{(Q - \text{df})}{Q} \times 100 \quad (7)$$

where df is degrees of freedom. The phenotypic variance attributed to a SNP was determined as follows:

$$V_p = 2p(1-p)\beta^2 \quad (8)$$

Where p is the frequency of effect allele and β is its regression coefficient derived from the association model (25). We calculated the statistical power of our discovery sample to detect SNPs with varying effect size (V_p) using a linear regression model. Power analysis was done in R (version 3.4.3) under the assumption of an additive mode of inheritance, $N = 551$, and different levels of α (type I error).

Transcriptional Reporter Assays

Specifically in skeletal muscle, the region encompassing rs679482 is DNase protected and predicted to bind DMRT2 (Supplementary Fig. 1).

A 504–base pair (bp) sequence (hg19 DNA range = chr13:23765249–23765748), encompassing rs679482, was amplified using primers (F-SGCGenhancerBam, 5′- GTAGGATCC CAAGACATAAATCATCTT -3′, and R-SGCGenhancerBam, 5′- ACAGGATCCTATGATGATCAACTCTG -3) and cloned into pGL3 promoter (pGL3p) vector. Two constructs were generated with respect to the genotype of rs679482. Each of C2C12 cell line and human primary skeletal muscles cells were cotransfected with the enhancer construct, HSV-thymidine kinase promoter, using Lipofectamine 3000 (Thermo Fisher Scientific). Luciferase assays were performed with cell lysates using the Dual-Luciferase Reporter Assay System (Promega) 24 h posttransfection. Firefly luciferase readings were normalized to Renilla luciferase readings and compared with empty vector.

DMRT2 Overexpression

The Pax3/Dmrt2/Myf5 regulatory cascade has a known role in myogenesis (26), and rs679482 is predicted to alter *DMRT2* binding affinity. *DMRT2* expression is low in skeletal muscle satellite cells compared with differentiated myotubes. The human *DMRT2* sequence along with hemagglutin (HA) tagged at the COOH-terminal was synthesized by Bio Basic gene synthesis service. The gene fragment was subcloned into mammalian expression vector pLVX. This vector, along with psPAX2 and pMD2.g, was used to generate *DMRT2*-expressing lentivirus. Luciferase assays were repeated following overexpression of *DMRT2* in human skeletal muscle satellite cells and C2C12 cells to determine effects of rs679482 on *DMRT2*-induced transcriptional activity.

Chromatin Immunoprecipitation

Two lots of each of rs679482 homogyzote (CC) and heterogyzote (CA) human skeletal muscle satellite cells were used in these experiments. *DMRT2* was transduced in human skeletal muscle satellite cells for 48 h, after which the cells were cross-linked in 1% paraformaldehyde at room temperature for 10 min. Cross-linking was quenched by adjustment to 125 mmol/L glycine, and the cells were collected by scraping in PBS followed by centrifugation at 1,000*g* for 10 min at 4°C. Cells were then lysed in chromatin immunoprecipitation (ChIP) buffer (50 mmol/L Tris-HCl, pH 8.0; 150 mmol/L NaCl; 1 mmol/L EDTA, pH 8; 1% Triton X-100; 0.1% sodium deoxycholate; 0.1% SDS; and complete protease inhibitors) (Roche Diagnostics). Genomic DNA was sheared to an average fragment size of 200–300 bp by sonicating the cell lysates in a Bioruptor (Diagenode). Prewashed anti-HA magnetic beads (Pierce) were used to capture DMRT2-bound DNA, which was eluted in ChIP elution buffer (1% SDS, 100 mmol/L $NaHCO_3$). Protein-DNA cross-links were reversed by incubating at 65°C for 5 h in the presence of 0.2 mol/L NaCl. DNA was further purified using a PCR cleanup kit (FroggaBio). Quantification of the DNA fragments harboring rs679482 was determined by quantitative PCR using primers surrounding rs679482; fold enrichment is calculated relative to the input. The percentage of each allele in the recovered DNA was assessed using a standard curve that was generated by measuring the ratio of each TaqMan probe signal as a function of allele proportion.

Data and Resource Availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

RESULTS

Heritability of Weight Loss

We used the GREML algorithm implemented in GCTA software to estimate the sum contribution of all SNPs (N = 4,681,082 SNPs) to variance in weight loss. We found genome-wide SNPs (MAF >0.01) explain 49% of phenotypic variance in weight loss response to dietary restriction program (Table 1).

Analysis of 20 sex-matched sib-pairs having completed the program with 112 age- and sex-matched unrelated individuals also demonstrated that siblings exhibit much greater similarity of weight loss in response to the behavioral intervention compared with unrelated individuals ($P = 0.007$) (Supplementary Fig. 2). Taken together, these findings indicate weight loss in response to energy restriction is a heritable trait with heritability within the range reported in a recent meta-analysis for heritability estimates of obesity (40%–75%) (27).

Clinical Determinants of Weight Loss

The discovery and replication samples were similar with regard to baseline characteristics including sex distribution, age, weight, and height of participants ($P < 0.05$, two-sample t test) (Table 2). The joint effect of baseline characteristics on the rate of weight loss was calculated by fitting a linear regression model as follows:

$$\text{26-week PWL} \sim \text{sex} + \text{age} + \text{initial weight} + \text{height, respectively}$$

Table 1—Heritability of weight loss explained by common genome-wide SNPs

Parameter	Description	Variance	SE
V_g	Genetic variance	0.48	0.25
V_e	Environmental variance	0.50	0.25
V_p	Phenotypic variance	0.99	0.04
h^2	Heritability (95% CI)	0.49 (0–0.98)	0.25
P	P value	2.23×10^{-2}	
N	Sample size	108	

Male participants exhibited greater 26-week PWL after age, initial weight, and height differences were taken into account ($\beta = 0.02$, $P = 8 \times 10^{-7}$). PWL decreased with age ($\beta = -3E\text{-}4$, $P = 0.02$) (Supplementary Table 1). As such, we adjusted the weight loss phenotype for age and sex using linear regression and used the derived residuals to carry out the GWAS analysis.

GWAS

IBS pruning (proportion of genetic resemblance) >0.05 retained a subset of 551 unrelated individuals in the discovery cohort (Supplementary Fig. 3). As shown in Supplementary Fig. 4*A*, study subjects clustered within the European sample and the first 10 principal components were used to control for subtle population structure (Supplementary Fig. 4*A–C*). The power of our study to detect SNPs with different effect sizes (V_p) is shown in Supplementary Fig. 5, indicating adequate power (power ≥80%) to detect SNPs with V_p (attributed phenotypic variance) as small as 1% at $\alpha = 0.05$ and as small as 8% at genome-wide significance level ($\alpha = 5e^{-8}$). The quantile-quantile plot of our GWAS analysis is provided in Supplementary Fig. 4*D*. Genomic inflation (λ) = 1.01, indicating that the GWAS was not affected by population structure, cryptic relatedness, or unknown errors in genotyping and imputation steps.

No SNP reached a genome-wide significance threshold ($P = 5 \times 10^{-8}$) in the discovery sample (Supplementary Fig. 6). This was expected, given our small sample size ($n = 551$) and lack of statistical power (Supplementary Fig. 5) to detect SNPs with small effect sizes at $\alpha = 5 \times 10^{-8}$. Therefore, we selected independent SNPs ($r^2 < 0.2$) with $P < 5 \times 10^{-5}$ from our discovery sample ($N = 14$ SNPs) (Supplementary Table 2) and tested their association in our exploratory sample. Only SNP rs679482 (intronic to *SGCG*) showed a concordant significant association in the exploratory sample (Supplementary Table 2). The regional association plot appears in Supplementary Fig. 7. rs679482 was imputed in both our discovery (imputation INFO = 0.95) and exploratory (imputation INFO = 0.90) sample. As such, we genotyped all subjects ($N = 710$) from our exploratory sample and an additional 621 subjects using a TaqMan assay (total sample = 1,331). We found rs679482 to be highly associated with weight loss in the replication sample of 1,331 subjects ($P = 1.2 \times 10^{-8}$) (Table 3) where rs679482 was directly genotyped.

The minor allele (A) was concordantly associated with less weight loss in both discovery and replication samples and surpassed the GWAS significance threshold in the combined meta-analysis ($P = 1.7 \times 10^{-12}$) (Table 3 and Fig. 1) with no evidence of between-study heterogeneity ($I^2 = 0$, Cochran Q test P value = 0.7). Of note, rs679482 explained 3% of weight loss variance (V_p, or phenotypic variance) in the discovery sample, 2.3% of variance in the replication sample, and 2.6% of phenotypic variance in combined meta-analysis. As presented in Supplementary Table 3, rs679482 was in Hardy-Weinberg equilibrium in the three cohorts.

To investigate whether the effect of rs679482 on weight loss differs in males versus females, we fitted a linear regression model: weight loss ~ sex + age + rs679482 + rs679482:sex, where rs679482:sex is rs679482-sex interaction.

We did not find a significant rs679482-sex interaction ($P > 0.3$) with regard to weight loss in either the discovery ($P = 0.5$) or replication ($P = 0.3$) sample (Supplementary Table 4).

Table 2—Baseline characteristics of study subjects

Discovery sample	Female: $N = 402$	Male: $N = 149$	All: $N = 551$
26-week PWL	19.45 ± 0.26	21.61 ± 0.52	20.04 ± 0.24
Height (in)	64.4 ± 0.12	70.0 ± 0.23	65.9 ± 0.15
Initial weight (lb)	254.1 ± 2.4	320.0 ± 5.5	271.9 ± 2.6
Age (years)	45.6 ± 0.54	46.2 ± 0.80	45.8 ± 0.45
Initial BMI (kg/m^2)	43.1 ± 7.7	45.9 ± 9.2	43.8 ± 8.2
Waist circumference (cm)	116.9 ± 15.3	139.4 ± 17.1	123.1 ± 18.7
Replication sample	Female: $N = 963$	Male: $N = 368$	All: $N = 1,331$
26-week PWL	18.04 ± 0.18	20.51 ± 0.35	18.72 ± 0.17
Height (in)	64.4 ± 0.08	70.0 ± 0.14	65.9 ± 0.10
Initial weight (lb)	256.1 ± 1.5	310.9 ± 3.0	271.3 ± 1.5
Age (years)	46.5 ± 0.35	48.1 ± 0.57	46.9 ± 0.30
Initial BMI (kg/m^2)	43.6 ± 7.8	44.8 ± 8.2	44 ± 7.9
Waist circumference (cm)	117.8 ± 14.2	138.3 ± 16.5	123.5 ± 17.4

Table 3—Association of rs679482 with 26-week PWL in discovery, replication, and combined meta-analysis

Study	*N*	A1	A2	Freq	β (95% CI)‡	SE	*P*	V_p§
Discovery	521	A	C	0.12	−0.38 (−0.56, −0.20)	0.09	3.7E-05	3.0
Replication	1,331	A	C	0.11	−0.34 (−0.46, −0.22)	0.06	1.2E-08	2.3
Meta-analysis	1,852	A	C	0.12	−0.35 (−0.45, −0.25)	0.05	1.7E-12	2.6

Freq, frequency of A1 allele. ‡95% CI around regression coefficient, β. §Percentage of phenotypic variance explained by rs679482.

PRS Analysis

In an earlier study, we performed a phenome-wide search for traits that are causally associated with the risk of cardiovascular disease (23). Here, we tested whether these traits are also associated with the weight loss success. After correcting our results for multiple testing, we found that the association between markers of body adiposity and weight loss remains significant (Table 4 and Supplementary Table 5). These findings indicate that subjects genetically predisposed to greater adiposity tend to lose less weight during the controlled dietary intervention program.

rs679482 Shows Allelic Differences in Enhancer/Repressor Activity and DMRT2 Protein Binding

The GWAS-identified lead SNP, rs679482 is not closely linked ($r^2 < 0.4$) to other variants, and HaploRegv4 data indicate that this region is DNase protected and rs479682 alters DMRT2, HNF1, and Pou3f2 motifs in muscle. We next examined each of the rs679482 alleles in the sequence in both orientations with respect to a minimal promoter in C2C12 cells and human skeletal muscle satellite cells (Fig. 2*A*). As shown In Fig. 2*B*, the sequence containing rs679482 demonstrated repressor activity relative to pGLP3 promoter (pGLP3p) but repressor activity was significantly attenuated for the construct containing the alternative allele (A) in both C2C12 cells (A_{fwd} vs. C_{fwd} $P = 4.9 \times 10^{-20}$) and HSM cells (A_{fwd} vs. C_{fwd} $P = 0.04$).

We then overexpressed *DMRT2* in each of the cell types and repeated luciferase assays. In the presence of *DMRT2* as shown in Fig. 2*C*, the sequence containing this SNP demonstrated an approximately twofold increase in enhancer activity relative to pGLP3p, with greater enhancer activity for the construct containing the alternative allele

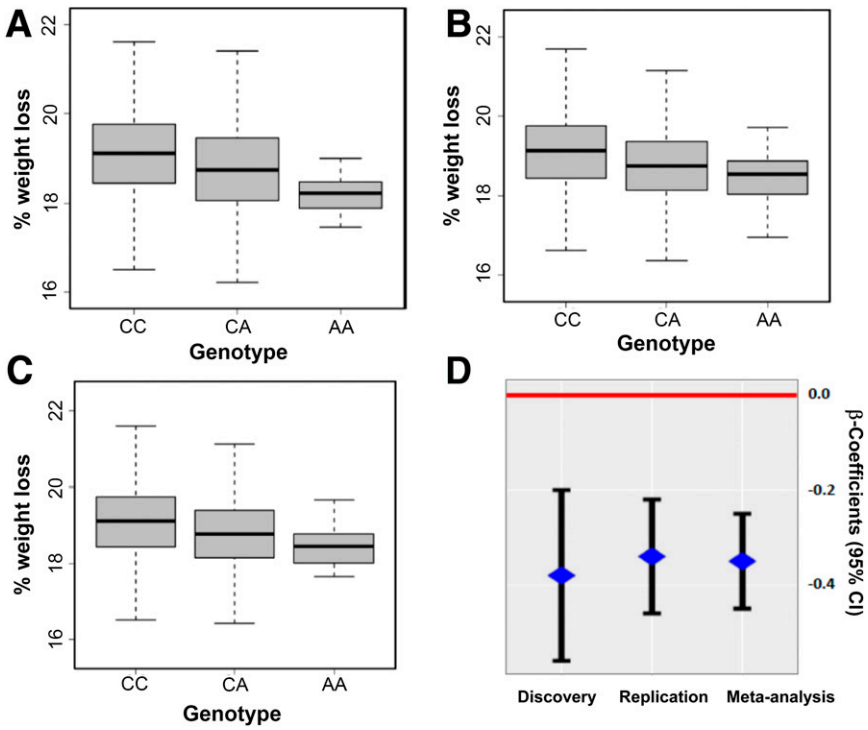

Figure 1—Association of weight loss with rs679482. The box plots depict the distribution of % weight loss by genotype in discovery (*A*), replication (*B*), and combined (*C*) samples. Association between rs679482 and % weight loss has an additive mode of inheritance, where subjects with A allele tend to lose less weight compared with subjects with C allele. *D*: The forest plot depicts the effect size and direction of association (based on A allele) in discovery and replication and the combined meta-analysis. The diamonds and the black vertical lines represent β regression coefficients and 95% CIs. Of note, rs679482 explained 3% of weight loss variance (V_p) in the discovery sample, 2.3% of variance in the replication sample, and 2.6% of phenotypic variance in combined meta-analysis.

Table 4—PRS analysis: genetic risk for measures of adiposity associates with impaired weight loss success*

	GWAS data source	β	SE	*Z*	*P*
Leg fat mass	UKBB	−6.8E-03	1.7E-03	−3.923	9.28E-05
Whole-body fat mass	UKBB	−6.8E-03	1.7E-03	−3.887	0.000108
Leg fat percentage	UKBB	−6.6E-03	1.7E-03	−3.803	0.00015
Trunk fat percentage	UKBB	−6.4E-03	1.7E-03	−3.64	0.000285
Body fat percentage	UKBB	−6.3E-03	1.7E-03	−3.606	0.000324
Trunk fat mass	UKBB	−6.2E-03	1.7E-03	−3.536	0.000423
BMI	UKBB	−6.1E-03	1.7E-03	−3.528	0.000435
Arm fat mass	UKBB	−6.1E-03	1.7E-03	−3.513	0.000461
Arm fat percentage	UKBB	−5.9E-03	1.7E-03	−3.411	0.000669

UKBB, UK Biobank. *The complete version of this table for all tested traits is available as Supplementary Table 5.

(A) in both C2C12 cells (A_{fwd} vs. C_{fwd} $P = 7.7 \times 10^{-9}$) and HSM cells (A_{fwd} vs. C_{fwd} $P = 2.8 \times 10^{-4}$).

ChIP analysis demonstrates that the region encompassing rs679482 is 2.68-fold enriched in human skeletal muscle satellite cells overexpressing *DMRT2* ($P = 0.04$, paired two-tailed *t* test) (Fig. 3*A*). Allelic preference for rs679482-A is evident in the recovered ChIP DNA, with 58% of allele (A) and 42% of allele (C) recovered from heterozygote HSM cells ($P = 0.03$, two-tailed *t* test) (Fig. 3*B*).

The region encompassing rs679482 bears the chromatin mark H3K36me3 that is associated with transcription elongation and splicing (28). GTEx data demonstrate that rs679482 is a splicing quantitative trait locus in heart muscle, with an increased intron-to-excision ratio in carriers of the A allele that is associated with impaired weight loss success ($n = 386$; $P = 2.8 \times 10^{-12}$).

DISCUSSION

Recent large GWAS have identified >500 common variants associated with BMI, body fat distribution, and related anthropometric traits (29). However, limited data are available on the genetic architecture of variability in weight loss in response to diet, despite evidence that this is a heritable trait (5,9). In this study, we find that first-degree relatives exhibit significantly greater concordance in weight loss success in response to diet compared with unrelated or cohabitating individuals (Supplementary Fig. 2). Furthermore, SNP-based analysis indicates weight loss is a heritable trait ($h^2 = 0.49$) (Table 1), with a heritability within the range reported for obesity (40%–75%) (27). Given the small sample sizes available for both sib-pair and SNP-based analysis, future studies using larger data sets, as these become available, will be required.

To our knowledge, this is the first GWAS for weight loss variability in response to a 26-week controlled dietary regimen. Here, we identify in a GWAS sample consisting of 551 highly compliant individuals, with replication in an independent cohort of 1,331 individuals, the novel association of a common intronic SNP, rs679482C>A in *SGCG* (γ-sarcoglycan), with weight loss in response to diet. Although no other locus reached genome-wide significance, we found that PRS related to measures of body adiposity associate with impaired weight loss in response to energy restriction (Table 4). Consistently, de Toro-Martín et al. (30) reported that individuals with a higher BMI PRS exhibited decreased weight loss success after biliopancreatic diversion with duodenal switch. Bandstein et al. (31) found that patients in the lowest quartile of a BMI PRS were more successful at losing excess weight after RYGB. Svendstrup et al. (32) reported that a genetic risk score associated with greater waist-to-hip ratio was associated with impaired diet-induced weight loss in women. Of note, we did not find a significant association between *FTO* SNPs and weight loss. This is consistent with the findings of a recent systemic review and meta-analysis of data of 9,563 individual participants from eight randomized controlled trials of response to weight loss intervention (33).

We find that male participants lose weight more readily than females (Supplementary Table 1). It is possible to systematically investigate whether this is explained by sex difference in responses to genetic risk by dividing the sample by sex and calculating the genetic correlation with weight loss in males and females. We could not carry out this analysis in our discovery sample due to lack of statistical power. However, we previously found that the differences between males and females with regard to BMI are not due to common autosomal genomic variants (22). Furthermore, although sex was a highly significant factor in our association model ($P < 0.0001$) (Supplementary Table 1), the effect of rs679482 on weight loss was not different between males and females in either discovery or replication samples ($P > 0.3$) (Supplementary Table 4).

The majority of GWAS signals for important metabolic traits have not been functionally investigated. Such analyses can add importantly to our understanding of the biology underlying a variety of complex phenotypes. In this study, we show evidence that rs679482 is a regulatory variant in the newly identified *SGCG* locus for weight loss success. Specifically, in skeletal muscle, the region encompassing rs679482 is DNase protected and bears the enhancer chromatin mark H3K4me1 (28). The rare allele

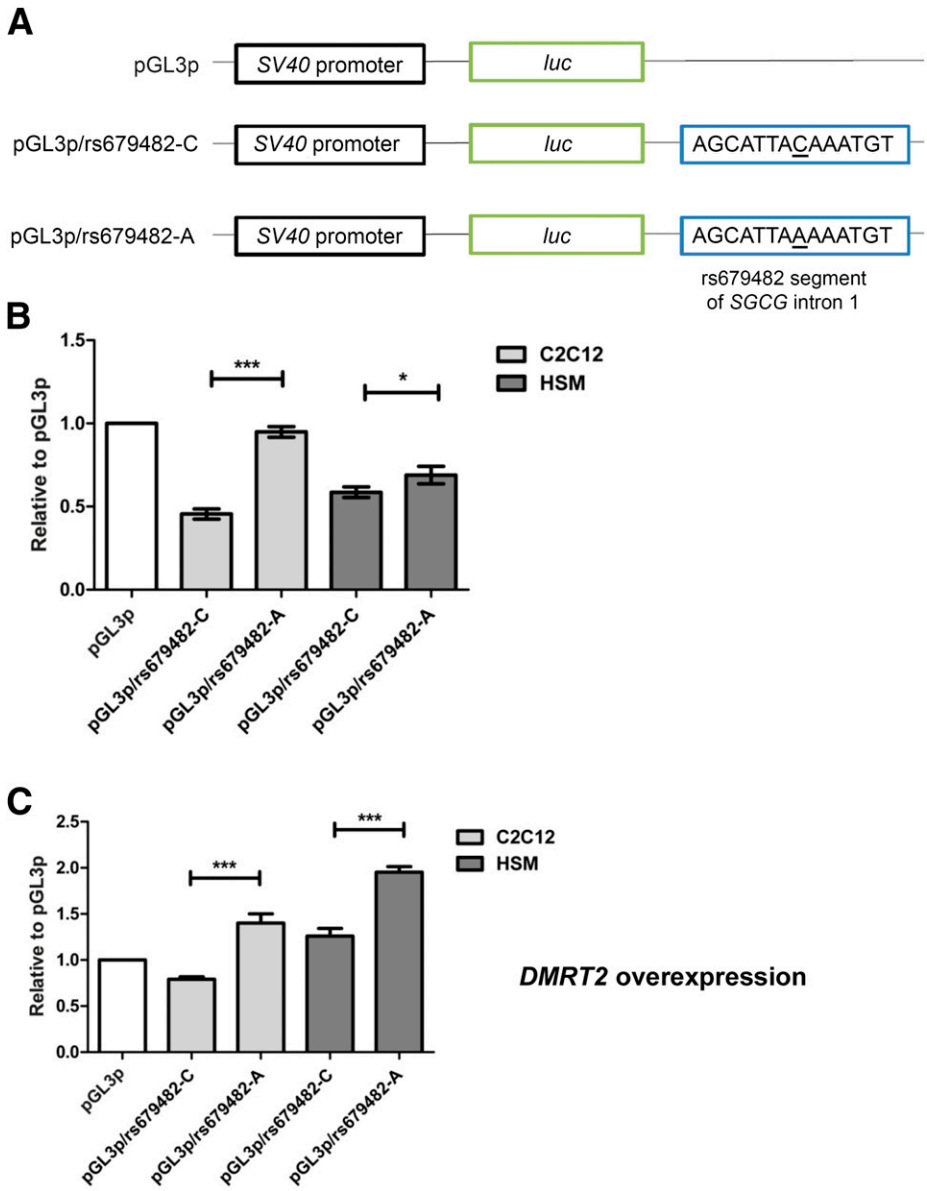

Figure 2—Transcriptional reporter assays. *A*: We examined each of the rs679482 alleles in the sequence in both orientations with respect to a minimal promoter in C2C12 cells and human skeletal muscle satellite cells. *B*: the sequence containing rs679482 demonstrated repressor activity relative to pGLP3p but with diminished repressor activity for the construct containing the alternative allele (A) in C2C12 cells (A_{fwd} vs. C_{fwd} $P = 4.9 \times 10^{-20}$) and HSM cells (A_{fwd} vs. C_{fwd} $P = 0.04$). *C*: Since rs679482 overlaps a DMRT2 binding site in skeletal muscle, we next overexpressed *DMRT2* in each of the cell types and repeated luciferase assays. With the presence of *DMRT2*, the sequence containing this SNP demonstrated an approximately twofold increase in enhancer activity relative to pGLP3p but with greater enhancer activity for the construct containing the alternative allele (A) in C2C12 cells (A_{fwd} vs. C_{fwd} $P = 7.7 \times 10^{-9}$) and HSM cells (A_{fwd} vs. C_{fwd} $P = 2.8 \times 10^{-4}$). luc, luciferase.

rs679482-A strongly associates with impaired weight loss response in obese individuals. By allele-specific transcription factor binding analysis, we show preferential binding of DMRT2 to rs679482-A. Consistently, the rs679482-A risk allele exhibits higher transcriptional activity alone and in response to *DMRT2* overexpression in human skeletal muscle and C2C12 cells.

SGCG encodes one of the sarcolemmal transmembrane glycoproteins that interact with dystrophin. The dystrophin-glycoprotein complex spans the sarcolemma and thus provides a structural link between the subsarcolemmal cytoskeleton and the extracellular matrix of skeletal myocytes. SGCG participates in binding actin to the extracellular matrix of skeletal and cardiac muscle cells and is involved in contractility. Rare mutations and deletions in *SGCG* associate with early-onset autosomal recessive limb-girdle muscular dystrophy, type 2C (34,35).

As a component of the sarcoglycan complex, SGCG is important for muscle development and integrity. In a robust analysis of the insulin sensitivity transcriptome using

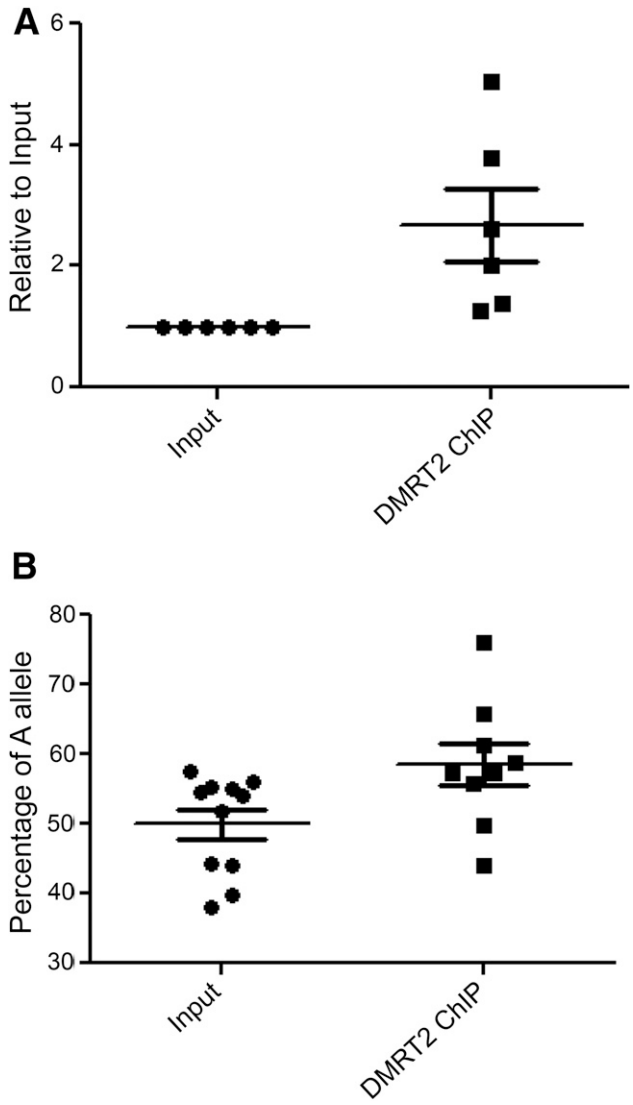

Figure 3—Allele-specific DMRT2 binding. rs679482 is located within a predicated DMRT2 binding site in skeletal muscle. *A*: ChIP demonstrates that the region encompassing rs679482 is 2.68-fold enriched in human skeletal muscle satellite cells overexpressing *DMRT2* ($P = 0.04$, paired two-tailed *t* test). *B*: An allelic differential preference is evident among the recovered ChIP DNA, with 58% of allele (A) and 42% of allele (C) recovered from heterozygote HSM cells ($P = 0.03$, two-tailed *t* test).

1,012 skeletal muscle samples, Timmons et al. (36) identified *SGCG* as 1 of 16 core insulin sensitivity genes that responded to four independent exercise-training interventions. Increased *SGCG* expression in response to exercise associated with improved insulin sensitivity. This may reflect an increase in muscle mass that would also be expected to have positive effects on energy expenditure.

Publicly available GWAS data reveal that this SNP is primarily associated with metabolites and that the top associated trait for this SNP is BMI (Supplementary Table 6). Fine mapping of a quantitative trait locus on chromosome 13 for submaximal exercise capacity training response ($VO_2$60) in the HERITAGE (HEalth, RIsk factors, exercise Training And GEnetics) Family Study narrowed the target region to two candidate genes, *MIPEP* and *SGCG* (37). An unlinked SNP in *SGCG*, rs9552911, was associated with type 2 diabetes in a South Asian population (38). Furthermore, in a group of 1,972 Chinese individuals, comprising 966 patients with type 2 diabetes and 976 control subjects, the same SNP was associated with type 2 diabetes ($P = 0.017$) and obesity (BMI $\geq$28 vs. BMI $<$28 kg/m^2) ($P = 0.033$) (39). However, rs9552911 could not be interrogated in the current study, since it has an allele frequency of $<$0.01 in Europeans compared with 0.23 in the Asian population.

Other loci linked to weight loss success include a 15q26.1 locus near *ST8SIA2* and *SLCO3A1* identified in a study of RYGB patients (6) and a copy number variant in the amylase gene cluster in a study of diet response (7). We have taken great care to remove from study individuals who did not adhere to the intervention or harbored medical conditions contributing to apparent diet resistance. This is an imperfect endeavor, and we acknowledge that compliance remains a partial confounder in human investigations such as this.

In summary, we demonstrate that weight loss success is a heritable trait with a heritability estimate similar to that of obesity. We identify a novel signal rs679482 at the *SGCG* locus that reaches genome-wide significance for weight loss success and provide evidence of a potential molecular mechanism by which this variant leads to impaired diet response.

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