Human Cardiovascular Disease IBC Chip-Wide Association with Weight Loss and Weight Regain in the Look AHEAD Trial

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Key Words

Type 2 diabetes · Obesity · Weight loss · Diet · Genetics

Abstract

Background/Aims: The present study identified genetic predictors of weight change during behavioral weight loss treatment. **Methods:** Participants were 3,899 overweight/obese individuals with type 2 diabetes from Look AHEAD, a randomized controlled trial to determine the effects of intensive lifestyle intervention (ILI), including weight loss and physical activity, relative to diabetes support and education, on cardiovascular outcomes. Analyses focused on associations of single nucleotide polymorphisms (SNPs) on the Illumina CARE iSelect (IBC) chip (minor allele frequency >5%; n = 31,959) with weight change at year 1 and year 4, and weight regain at year 4, among individuals who lost ≥3% at

year 1. **Results:** Two novel regions of significant chip-wide association with year-1 weight loss in ILI were identified ($p < 2.96 \times 10^{-6}$). *ABCB11* rs484066 was associated with 1.16 kg higher weight per minor allele at year 1, whereas *TNFRSF11A*, or *RANK*, rs17069904 was associated with 1.70 kg lower weight per allele at year 1. **Conclusions:** This study, the largest to date on genetic predictors of weight loss and regain, indicates that SNPs within *ABCB11*, related to bile salt transfer, and *TNFRSF11A*, implicated in adipose tissue physiology, predict the magnitude of weight loss during behavioral intervention. These results provide new insights into potential biological mechanisms and may ultimately inform weight loss treatment.

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Introduction

Obesity is a major public health problem [1]. Fully 64% of the US population is estimated to be overweight or obese (body mass index ≥ 25) [2] and is at increased risk for weight-related comorbidities, including coronary artery disease, diabetes and certain cancers. Behavioral weight loss intervention, focusing on changes in diet and physical activity, has emerged as a key strategy in combating this rise in obesity and the associated health consequences [3, 4]. These weight loss programs often produce initial weight losses of $\geq 7\%$, resulting in clinically important health benefits [5, 6]. Nonetheless, partial weight regain is common [6]. A greater understanding of predictors of weight loss and weight maintenance or regain could have important health benefits.

Obesity is also a quintessential phenotype to study the interplay of genetic and environmental factors. Body weight is well known to be heritable [7, 8] and obesity susceptibility loci have been identified through genome-wide association studies (GWAS), although the variance attributable to these loci remains small [9–11]. At the same time, the dramatic rise in obesity rates over the past 30 years suggests environmental influences [12] and evidence of gene $\times$ environment interaction can be derived from both twin [13, 14] and molecular genetic studies [15, 16].

Randomized controlled trials (RCTs) comparing behavioral weight loss treatment to a control condition afford a unique opportunity to test gene $\times$ environment interaction in the context of gene $\times$ treatment arm interaction [17]. Participants are randomly assigned to a treatment that promotes successful weight loss through provision of caloric and physical activity goals and teaching of behavioral strategies, or a control treatment without active weight loss. One type of gene $\times$ environment interaction may occur if genetic markers are associated with weight change in a control arm but not a behavioral weight loss treatment arm, suggesting that behavioral weight loss treatment may mitigate genetic effects on the outcome. Alternatively, a gene $\times$ environment interaction could occur if genetic markers relate to weight change in the intervention arm but not the control arm, suggesting that genetic factors may influence the ability to lose weight. Both effects are of potential public health importance as they may identify genetic predictors of naturalistic weight gain over time and/or resistance to weight loss. These effects further have potential clinical application as they are examined in the context of a well-established treatment paradigm. It is important to note, none-

theless, that although RCTs with longitudinal follow-up afford many advantages in research design and treatment implications, implementation of such a trial is costly and complex and sample sizes are necessarily smaller than those common in epidemiologic studies of gene $\times$ environment interaction.

The goal of the present study is to conduct a chip-wide association study of nearly 32,000 single nucleotide polymorphisms (SNPs) available from the Illumina CARE iSelect (IBC) chip [18] with weight loss at year 1 and year 4 in response to behavioral weight loss intervention and weight change at year 4 among those who lost $\geq 3\%$ weight at year 1. The Look AHEAD study, the largest RCT comparing behavioral weight loss to a control condition with an effective intervention and excellent longitudinal follow-up, provides an excellent opportunity to conduct such analyses.

Materials and Methods

Study Cohort

The Look AHEAD study enrolled 5,145 ethnically diverse overweight and obese subjects with type 2 diabetes and aged 45–76 years. Of these, 1,108 were excluded for lack of consent for genetics studies, lack of institutional review board approval for this ancillary study (including the Southwest American Indian sites), 10 for withdrawn consent for genotyping, and 60 for inadequate DNA samples. This left 4,037 individuals, of which 3,899 contributed genetic data that passed genotyping quality control procedures. These subjects form the basis for the present analyses.

The design and methods of the Look AHEAD trial have been reported elsewhere [19], as have the baseline characteristics of the randomized cohort [20]. Briefly, at baseline, participants were randomized to either an intensive lifestyle intervention (ILI) or a diabetes support and education (DSE) arm. Both the ILI and DSE groups were provided one session of education on diabetes and cardiovascular risk factors. In addition, ILI participants received an intensive lifestyle program, combining diet modification and increased physical activity, designed to produce an average of 7% weight loss and maintain this weight loss. The ILI included 1 individual and 3 group meetings per month for 6 months followed by 1 individual and 2 group meetings per month through 1 year. From years 2 to 4, ILI participants were seen individually at least once a month, contacted another time each month by telephone or e-mail, and offered a variety of ancillary classes. ILI sessions focused on behavioral weight loss strategies, such as self-monitoring, goal setting and stimulus control, to achieve and maintain weight loss. The DSE group received the option of attending 3 sessions per year on nutrition, physical activity and social support with no explicit weight loss goals. In the full trial [6, 21], maximal difference in average weight loss across intervention arm occurred at 1 year follow-up (8.6% in ILI vs. 0.7% in DSE, $p < 0.001$), with an average weight loss of 4.7% in ILI and 1.1% in DSE at year 4 follow-up. The Look AHEAD trial, including genetic analyses, was approved by local institutional review boards.

Anthropometric Measures

Weight was measured to the nearest 0.1 kg in duplicate at baseline, and year 1 and year 4 follow-ups, using a digital scale. Height was measured in centimeters at baseline using a standard wall-mounted stadiometer. Participants wore light clothing or a hospital gown and removed their shoes.

Weight regain was defined as weight change from year 1 to 4 among individuals initially losing at least some weight ($\geq 3\%$) at year 1 following methods used in the Diabetes Prevention Program [22]. As can be seen in online supplementary table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000353181), among those who lost $\geq 3\%$ weight at year 1, women regained 3.7 ± 8.2 kg and men regained 4.8 ± 7.8 kg from year 1 to 4 on average. It is important to note, however, that only 72.5% of women and 78.5% of men in this subgroup regained weight, as defined by a weight at year 4 greater than their weight at year 1, while the remaining individuals either maintained or continued to lose weight.

Genotyping

The genomic DNA extraction was based on the use of FlexiGene DNA Kit (Qiagen Inc., Valencia, Calif., USA) as described by the manufacturer, and DNA quantitation was performed using the PicoGreen dsDNA Quantitation Reagent (Invitrogen, Inc., Carlsbad, Calif., USA). Genotyping was carried out at the Children's Hospital of Philadelphia using the IBC chip, a gene-centric 50,000 SNP array designed to assess relevant loci across a range of cardiovascular, metabolic and inflammatory syndromes [18]. SNPs were clustered into genotypes using the Illumina Beadstudio software and subjected to quality control filters. Individual samples were excluded for individual call rates $<95\%$, gender mismatch, and duplicate discordance. SNPs were removed for call rates $<95\%$. To facilitate model convergence and minimize the penalty from multiple comparisons due to the many low-frequency SNPs included in the design, we filtered out markers with minor allele frequency (MAF) <0.05 . This left 31,692 autosomal SNPs on the IBC chip with MAF $>5\%$ whose mean genotyping success rate was 99.8%.

Observed genotype frequencies were compared with those expected under Hardy-Weinberg equilibrium (HWE) using stratified χ^2 tests within the two largest racial/ethnic groups (non-Hispanic White and African-American). As the sample is selected for overweight and diabetes, we did not exclude SNPs based on deviation on a chip-wide basis from HWE, but reviewed individual SNP associations to ensure SNPs showing significant associations did not deviate from HWE.

Statistical Analysis

After pruning of SNPs in linkage disequilibrium (LD; $r^2 > 0.3$), the Eigenstrat algorithm [23], as implemented in Golden Helix version 7.1 (Bozeman, Mont., USA), was used to compute principal components for use as covariates to control for ancestry in the regression analyses. Principal component analysis results indicated that the majority of the variance among the multi-racial Look AHEAD cohort was accounted for by the first two principal components, which agreed with self-reported race/ethnicity in distinguishing Caucasians from African-Americans, and Hispanics from these other 2 groups [24]. Preliminary analyses indicated that additional principal components that would have better separated the Hispanic and Asian groups and helped identify the few Native American study participants did not contribute to the weight change models.

Consistent with the parent trial, we focused on weight (not body mass index) change (in kg) as the primary outcome. Therefore, we conducted longitudinal regression analysis of baseline, year-1 and year-4 weight measurements, modeled jointly as a tri-variate normal outcome with an unstructured covariance matrix. Three-way interaction models of individual SNP markers with measurement time (year 1 vs. baseline, year 4 vs. baseline) and study arm (ILI vs. DSE) were estimated in Splus 8.2 [25] using restricted maximum likelihood. An additive genetic model was used for all SNP markers, with genotype coded by the number of minor alleles (0/1/2 copies). Minor alleles and allele frequencies were determined from the entire sample of genotyped participants, i.e. race/ethnicity-specific allele frequencies were not used.

Therefore, four distinct types of SNP effects were estimated, all of which can be interpreted as the effect of one additional copy of the corresponding minor allele on (a) baseline weight within DSE (SNP main effect); (b) ILI-DSE differences in baseline weight (SNP $\times$ study arm interaction); (c) weight change within DSE (SNP $\times$ time interaction), and (d) ILI-DSE differences in weight change (SNP $\times$ time $\times$ study arm interaction). Effects specific to ILI or averaged over the ILI and DSE arms were subsequently obtained by changing the referent group for study arm and re-estimating the model. Of note, in a randomized trial one would expect no ILI-DSE differences in SNP effects on baseline weight levels, so set b of model parameters serves solely as a randomization check.

Longitudinal weight outcomes were additionally adjusted for study site, age, gender, and the first two ancestry informative marker principal components [24, 26]. Other than study site, all of these covariates were fully interacted with time, study arm, and time by study arm interaction, so as to allow for these covariate effects to vary across study arm and/or time point, in a manner similar to the SNP effects described above.

For chip-wide analyses, we calculated the effective number of uncorrelated markers among the 31,692 autosomal SNPs under investigation using the Li and Ji approach [27] and found it to equal just 17,254 after LD correction. A chip-wide significance threshold of $p = 2.97\text{E-}06$ would, therefore, be needed to control the more stringent familywise error rate criterion at the 5% level based on Sidak's multiplicity adjustment [28].

We also used a false discovery rate (FDR) approach to guide our reporting of *suggestive* (FDR $<20\%$) associations, operationalized via a rank ordering of the genetic markers according to their q values. FDR controls the expected proportion of incorrectly rejected null hypotheses among those deemed significant, rather than across the entire set of hypotheses being tested; this increases power relative to more stringent familywise error rate control at the cost of more type I errors. Calculated using the q value package of Dabney and Storey [29], q values are marker-specific quantities that represent the minimum FDR at which the corresponding hypothesis test could be declared significant [30]. q values preserve the same rank ordering of SNPs as that produced by p value calculations, but recalibrate them by the probability that they represent a false discovery. They were calculated separately for each coefficient of interest (ILI change, DSE change, pooled ILI and DSE and differential change).

We also examined the extent to which the genetic markers predicted weight regain at year 4 among those who lost $\geq 3\%$ of their initial weight at year 1. This method was previously used to characterize weight regain in the Diabetes Prevention Program [22]. The primary outcome for these analyses was SNP $\times$ time (year 1

Table 1. Population characteristics in the Look AHEAD genetic subcohort

Characteristic	Total (n = 3,899)	DSE (n = 1,964)	ILI (n = 1,935)
Women, n (%)	2,192 (56.2)	1,096 (55.8)	1,096 (56.6)
Ethnicity, n (%)			
African-American	618 (15.8)	305 (15.5)	313 (16.2)
American Indian ^a	20 (0.5)	9 (0.5)	11 (0.6)
Asian/Pacific Islander	41 (1.1)	19 (1.0)	22 (1.1)
Hispanic/Latino	307 (7.9)	159 (8.1)	148 (7.7)
Non-Hispanic White	2,835 (72.7)	1,430 (72.8)	1,405 (72.6)
Other (multiple)	78 (2.0)	42 (2.1)	36 (1.9)
Age, years	59.1±6.8	59.2±6.8	59.0±6.9
BMI			
Women	36.8±6.2	36.9±6.1	36.7±6.3
Men	35.3±5.5	35.1±5.2	35.5±5.8
Waist circumference, cm			
Women	111.4±13.7	111.5±13.6	111.3±13.8
Men	118.8±13.4	118.5±12.9	119.2±13.9
Weight at baseline, kg			
Women	96.7±17.5	96.6±17.4	96.8±17.7
Men	109.6±18.5	109.4±17.8	109.8±19.2
Weight at year 1, kg			
Women	92.1±17.8	95.6±17.5	88.7±17.3
Men	104.1±18.9	108.7±17.9	99.4±18.8
Weight at year 4, kg			
Women	93.3±17.8	94.4±17.7	92.3±17.9
Men	106.1±19.1	108.4±18.2	103.7±19.7
Weight change baseline – year 1, kg			
Women	–4.6±7.1	–0.9±5.1	–8.1±7.1
Men	–5.6±8.4	–0.9±5.2	–10.5±8.3
Weight change baseline – year 4, kg			
Women	–3.3±9.0	–2.2±9.4	–4.5±8.5
Men	–3.3±8.8	–0.9±7.8	–5.8±9.0

Values are means ± SD unless otherwise indicated. ^a The number of American Indian participants included in this ancillary study is less than that of the parent Look AHEAD trial because not all centers participated.

vs. year 4) interaction with the same covariates as above in addition to baseline weight. Sample size for this subanalysis was n = 2,022 (1,545 in ILI and 477 in DSE). All analyses were performed at Brown University.

Results

Descriptive Statistics

Participant characteristics of the subcohort of Look AHEAD used in these analyses are shown in table 1. Individuals were evenly distributed between the ILI and DSE intervention arms and had comparable age and gender as in the entire cohort (data not shown). The number of American Indian participants included in this study is less than that of the parent Look AHEAD trial due to dif-

ferences in informed consent for genetic ancillary studies. No baseline differences in demographic or clinical characteristics across ILI and DSE were observed. Further, no between-arm differences in baseline means of the outcomes of interest were detected across genotypic groups for any of the markers under consideration ($p > 2.96\text{E-}06$). Similar to the larger Look AHEAD trial [21], individuals assigned to ILI lost significantly more weight at year 1 and 4 than those assigned to DSE.

Demographics and weight change patterns among individuals who lost $\geq 3\%$ of their weight at year 1 are depicted in online supplementary table 1. In this subset, individuals in ILI lost more weight at year 1 and regained more weight from year 1 to year 4. Overall, among those who lost $\geq 3\%$ at year 1, 59% regained weight in DSE and 80% in ILI.

Fig. 1. IBC chip-wide Manhattan plot for year-1 weight change in ILI (**a**) and DSE (**b**). Includes 31,692 autosomal SNPs with MAF >5% (n = 3,889).

Genetic Associations with Weight Loss at Year 1

Genetic associations of the full set of SNP markers with year-1 weight change in ILI and DSE are depicted in figure 1. The association of two loci with year-1 weight change in the ILI group exceeded chip-wide significance after correcting for chip-wide multiple comparisons ($p < 2.96\text{E-}06$). One intronic locus represented by two SNPs in high LD, rs484066 and rs569805, within *ABCB11*, showed the strongest association with year-1 weight loss (online suppl. table 2). These SNPs were associated with a 1.16 and 1.24 kg higher weight per minor allele at year 1, re-

spectively, suggesting that the minor allele was associated with resistance to weight loss. A third SNP, rs17069904, within *TNFRSF11A*, or *RANK*, also achieved chip-wide significance. This SNP was associated with a 1.70 kg lower weight per allele at year 1, suggesting that the minor allele was associated with greater weight loss. These SNPs had no significant effects in DSE ($p > 0.51$). The resulting SNP $\times$ treatment arm interactions for the lead SNP at each locus were *ABCB11* rs484066 interaction, $p = 3.98\text{E-}05$ (fig. 2), and *TNFRSF11A* rs17069904 interaction, $p = 1.57\text{E-}04$ (fig. 3).

Fig. 2. Interaction plot for *ABCB11* rs484066 in men (a) and women (b).

Fig. 3. Interaction plot for *TNFRSF11A* rs17069904 in men (a) and women (b).

An additional 131 SNPs showed a suggestive association with year-1 weight change (online suppl. table 2; FDR $q < 0.20$). Eight additional SNPs were in the *ABCB11* region and an additional 3 SNPs in the *TNFRSF11A* region. Regional plots depicting the association of the *ABCB11* and *TNFRSF11A* regions with year-1 weight change are presented in online supplementary figures 1 and 2, respectively.

No additional SNPs showed suggestive evidence for association with year-1 weight change in DSE (FDR $q > 0.99$), year-1 weight loss as averaged across treatment arms (FDR $q > 0.99$) or SNP $\times$ treatment arm interaction (FDR $q > 0.20$).

Genetic Associations with Weight Loss from Baseline to Year 4

SNPs with a suggestive association (FDR $q < 0.20$) with year-4 weight are presented in table 2. No SNPs showed suggestive association with weight change at year 4 in ILI (FDR $q > 0.20$) or SNP $\times$ treatment arm interaction (FDR $q > 0.20$). It is of note, however, that one of the SNPs identified for year-1 weight change, rs17069904, within *TNFRSF11A*, continued to show evidence of association with change in ILI at year 4 ($p = 0.0002$) and SNP $\times$ treatment arm interaction ($p = 0.0002$).

For year-4 weight change in DSE, in contrast, 17 SNPs were identified with suggestive association (FDR $q < 0.20$). The strongest two loci occurred within *IRF5* and *ITGAV*,

Table 2. SNPs associated with year-4 weight change (FDR $q < 0.20$)

SNP	Chr	Closest gene	Minor allele	MAF, % ^a	Effect	β^b	SE	p value	q value ^c
rs13242262	7	<i>IRF5</i>	T	40.45	DSE	1.414	0.303	3.00E-06	0.051
rs4233788	2	<i>ITGAV</i>	A	24.74	DSE	-1.510	0.325	3.35E-06	0.051
rs10229001	7	<i>IRF5</i>	G	39.43	DSE	1.379	0.303	5.33E-06	0.054
rs4788114	16	<i>LAT</i>	A	7.50	DSE	-2.371	0.538	1.05E-05	0.081
rs3911084	2	<i>ITGAV</i>	C	28.20	DSE	-1.347	0.316	2.06E-05	0.104
rs17166351	7	<i>IRF5</i>	A	49.47	DSE	-1.252	0.295	2.24E-05	0.104
rs11240089	1	<i>BCL9</i>	G	23.17	DSE	1.731	0.410	2.47E-05	0.104
rs4244559	15	<i>MEIS2</i>	G	33.54	DSE	1.253	0.298	2.72E-05	0.104
rs720475	7	<i>ARHGEF5</i>	A	23.46	DSE	-1.403	0.340	3.71E-05	0.126
rs12490899	3	<i>TGFBR2</i>	A	5.39	DSE	-2.735	0.667	4.15E-05	0.127
rs1874328	7	<i>IRF5</i>	G	39.45	DSE	-1.217	0.300	5.06E-05	0.141
rs7808907	7	<i>IRF5</i>	G	49.90	DSE	-1.164	0.289	5.84E-05	0.149
rs6695965	1	<i>BCL9</i>	G	23.71	DSE	1.616	0.412	8.84E-05	0.197
rs6434188	2	<i>ITGAV</i>	A	22.48	DSE	-1.349	0.345	9.21E-05	0.197
rs977483	5	<i>Intergenic</i>	G	8.80	DSE	2.019	0.520	1.05E-04	0.197
rs10505996	12	<i>ITPR2</i>	G	8.06	DSE	-2.137	0.551	1.07E-04	0.197
rs10760707	9	<i>TXNDC4</i>	T	15.95	DSE	1.597	0.413	1.10E-04	0.197
rs649526	15	<i>PLA2G4F</i>	A	36.96	Pooled	-0.936	0.216	1.43E-05	0.186
rs11165294	1	<i>TGFBR3</i>	A	11.01	Pooled	-1.378	0.327	2.59E-05	0.186
rs11063488	12	<i>Intergenic</i>	C	43.93	Pooled	0.901	0.214	2.68E-05	0.186
rs913059	1	<i>TGFBR3</i>	C	24.86	Pooled	0.998	0.239	2.92E-05	0.186
rs621560	15	<i>PLA2G4F</i>	A	41.23	Pooled	-0.874	0.210	3.24E-05	0.186
rs8114057	20	<i>HNF4A</i>	A	47.13	Pooled	-0.857	0.208	3.91E-05	0.186
rs11213865	11	<i>POU2AF1</i>	A	6.30	Pooled	-1.694	0.413	4.09E-05	0.186

Chr = Chromosome. ^a MAF in the full sample. ^b Effect of each copy of the minor allele on baseline to year-4 weight change (in kg) within DSE, and baseline to year-4 weight change (in kg) pooled across ILI and DSE arms. ^c Ranking based on q values (FDR <20%).

represented by 5 and 3 SNPs, respectively. Seven SNPs also showed a suggestive association with weight change as averaged across ILI and DSE (FDR $q < 0.20$). Two of these SNPs were within *PLA2G4F* and another 2 within *TGFBR3*.

Weight Change from Year 1 to 4 among Those Who Lost $\geq 3\%$ at Year 1

SNPs with a suggestive association (FDR $q < 0.20$) with weight change at year 4 among those who initially lost $\geq 3\%$ of their weight at year 1 are presented in table 3. No suggestive associations were observed within the ILI arm (FDR $q > 0.99$). For weight change within the DSE arm, 11 SNPs showed suggestive evidence of association (FDR $q < 0.20$). The closest genes for these SNPs included *FOXP1*, *GRB2*, *COL1A2*, *RARB*, *MMP13*, *JUN* and *C8orf49*. Eight SNPs showed suggestive evidence of a main effect on weight change across treatment arms (FDR $q < 0.20$). The closest genes for these SNPs were *FOXP1*, *MMP13*, *TGFBR3*, *C8orf49*, *FDFT1* and *ST8SIA4*.

For SNP $\times$ treatment arm interaction (table 4), 5 SNPs showed a suggestive association (FDR $q < 0.20$). Three of these SNPs occurred within *GRB2*, 1 within *TIMP3* and the last in the region of *INSR*.

Discussion

This paper presents the largest study and the first chip-wide analysis of weight loss in response to behavioral treatment and weight regain after successful weight loss. Our results identify novel regions of significant chip-wide association with magnitude of weight loss in response to behavioral treatment, including *ABCB11* and *TNFRSF11A* ($p < 2.96E-06$), as well as a number of suggestive associations (FDR $q < 0.20$) for weight loss at year 1, weight change from baseline to year 4, and weight regain at year 4 among those who lost $\geq 3\%$ at year 1. These results suggest new potential mechanisms contributing to

Table 3. SNPs associated with weight change from year 1 to 4 among participants who lost 3% weight by year 1

SNP	Chr	Closest gene	Minor allele	MAF, % ^a	Effect	β^b	SE	p value	q value ^c
rs830653	3	<i>FOXP1</i>	A	10.4	DSE	-3.894	0.843	3.98E-06	0.121
rs9302989	17	<i>GRB2</i>	G	26.79	DSE	2.964	0.680	1.33E-05	0.172
rs1800222	7	<i>COL1A2</i>	G	19.17	DSE	-3.052	0.711	1.79E-05	0.172
rs2053156	17	<i>GRB2</i>	C	27.67	DSE	2.762	0.659	2.82E-05	0.172
rs959260	17	<i>GRB2</i>	G	27.68	DSE	2.762	0.659	2.82E-05	0.172
rs17526019	3	<i>RARB</i>	A	6.67	DSE	-4.294	1.041	3.80E-05	0.178
rs10895373	11	<i>MMP13</i>	A	15.16	DSE	-3.008	0.733	4.10E-05	0.178
rs10895372	11	<i>MMP13</i>	G	15.41	DSE	-2.970	0.729	4.69E-05	0.179
rs2760494	1	<i>JUN</i>	G	16.02	DSE	-3.078	0.767	6.14E-05	0.189
rs11225490	11	<i>MMP13</i>	G	15.65	DSE	-2.892	0.723	6.45E-05	0.189
rs2740434	8	<i>C8orf49</i>	A	31.8	DSE	-2.321	0.582	6.82E-05	0.189
rs830653	3	<i>FOXP1</i>	A	10.4	Pooled	-2.208	0.493	7.65E-06	0.093
rs10895373	11	<i>MMP13</i>	A	15.16	Pooled	-1.807	0.415	1.37E-05	0.093
rs11225490	11	<i>MMP13</i>	G	15.65	Pooled	-1.780	0.409	1.40E-05	0.093
rs12567680	1	<i>TGFBR3</i>	A	32.53	Pooled	-1.325	0.305	1.40E-05	0.093
rs10895372	11	<i>MMP13</i>	G	15.41	Pooled	-1.790	0.413	1.48E-05	0.093
rs2740434	8	<i>C8orf49</i>	A	31.8	Pooled	-1.391	0.329	2.41E-05	0.113
rs1616534	8	<i>FDFT1</i>	G	46.36	Pooled	-1.274	0.304	2.76E-05	0.113
rs7703643	5	<i>ST8SIA4</i>	A	39.57	Pooled	-1.271	0.304	2.91E-05	0.113

Chr = Chromosome. ^a MAF in the full sample. ^b Effect of each copy of the minor allele on year-1 to -4 weight change (in kg) within DSE and as pooled across ILI and DSE arms. ^c Ranking based on q values (FDR <20%).

Table 4. SNPs associated with differential weight change (ILI–DSE) from year 1 to 4 among participants who lost 3% weight at year 1

SNP	Chr	Closest gene	Minor allele	MAF, % ^a	Effect	β^b	SE	p value	q value ^c
rs2053156	17	<i>GRB2</i>	C	27.67	Interaction	-3.506	0.751	3.14E-06	0.034
					ILI	-0.744	0.361	3.92E-02	
					DSE	2.762	0.659	2.82E-05	
rs959260	17	<i>GRB2</i>	G	27.68	Interaction	-3.504	0.751	3.21E-06	0.034
					ILI	-0.742	0.361	4.00E-02	
					DSE	2.762	0.659	2.82E-05	
rs713685	22	<i>TIMP3</i>	A	8.90	Interaction	4.858	1.045	3.43E-06	0.034
					ILI	1.895	0.500	1.53E-04	
					DSE	-2.963	0.917	1.25E-03	
rs9302989	17	<i>GRB2</i>	G	26.79	Interaction	-3.562	0.776	4.52E-06	0.034
					ILI	-0.598	0.374	1.10E-01	
					DSE	2.964	0.680	1.33E-05	
rs4804366	19	<i>INSR</i>	G	12.59	Interaction	4.039	0.957	2.50E-05	0.152
					ILI	0.881	0.444	4.72E-02	
					DSE	-3.158	0.848	1.99E-04	

Chr = Chromosome. ^a MAF in the full sample. ^b Effects of each copy of the minor allele on within-arm weight change for ILI and DSE as well as differential weight change (ILI–DSE) from year 1 to 4 (in kg). ^c Ranking based on q values (FDR <20%).

weight loss and regain and have the potential to inform behavioral weight loss treatment.

Prior genetic studies of weight loss had focused on variation within *PPARG* or obesity risk SNPs from GWAS. The Ala12Ala genotype in *PPARG* (rs1801282) has been associated with greater weight loss in response to lifestyle intervention both in the US Diabetes Prevention Program (DPP) [31] and the Finnish Diabetes Prevention Study (DPS) [32]. The association of *FTO* with weight loss is less clear. Obesity risk alleles within *FTO* predicted a greater increase in subcutaneous adipose tissue in the placebo group in the DPP [33] but greater free fatty mass in response to a low-protein diet and less free fatty mass in response to a high-fat diet in the POUNDS LOST trial [34]. In the DPP, the obesity risk allele at rs6265 in *BDNF* was also associated with greater weight regain over 2 years among those who had initially lost $\geq 3\%$ at 6 months [22]. In a prior Look AHEAD report [24], obesity risk SNPs derived from GWAS were not significantly associated with magnitude of initial weight loss. However, *FTO* risk alleles predicted weight regain in the DSE group, but not within the ILI group, resulting in SNP $\times$ treatment arm interaction. The obesity risk allele at *BDNF* rs6265 was also associated with marginally greater weight regain across treatment arms, consistent with the findings of the DPP.

In the present paper, we sought to broaden the prior literature by examining whether any additional SNPs represented on the IBC chip predict the extent of weight loss or weight regain during behavioral intervention. The IBC chip assays loci across a range of cardiovascular, metabolic and inflammatory syndromes [18], the majority of which had not previously been implicated in obesity or weight change. Thus, we could determine whether any of these regions might be relevant to weight-related phenotypes.

Genetic Predictors of Year-1 Weight Loss

For weight change at year 1, all SNP associations reaching chip-wide statistical significance or suggestive significance based on FDR occurred within ILI. This indicates that, at year 1, all of the SNP associations appear to influence ability to lose weight in response to intervention, whereas little to no effect of the SNPs was observed for naturalistic weight change in DSE over the year.

The strongest association with year-1 weight change in ILI was an intronic locus represented by 2 SNPs in high LD, rs484066 and rs569805, in *ABCB11*, or ATP-binding cassette, subfamily B, member 11, also called bile salt export pump (*BSEP*). *ABCB11* is the primary mediator of bile salt secretion across the canalicular membrane and

plays a critical role in absorption of dietary fat from the gut and counter transport of hepatic cholesterol from the liver to the intestine for elimination [35]. Mutations of *ABCB11* are known to cause progressive intrahepatic cholestasis [36, 37] and benign recurrent intrahepatic cholestasis [38, 39], and GWAS identify the *ABCB11* region as a predictor of alkaline phosphatase, a marker of biliary obstruction [40].

Perturbations of bile salt transport appear to alter body weight and serum cholesterol levels. *ABCB11* knockout mice have smaller body size than wild-type litter mates [41], while overexpression of *ABCB11* in mice leads to greater fat absorption from the intestine, more rapid weight gain and a reduction in energy expenditure in response to a high-fat diet but not on a control diet [42]. *ABCB11* variation has also been implicated in obesity in candidate gene studies [43], and, in GWAS, in fasting high-density lipoprotein and glucose [44]. It is of note that this locus is also proximal to glucose-6-phosphatase, catalytic, 2 (*G6PC2*), associated with fasting glucose in GWAS [45].

The *TNFRSF11A*, or as more commonly known *RANK*, polymorphism rs17069904 was also associated with weight loss at year 1 in response to ILI and nominally associated with year-4 weight change, with nominal SNP $\times$ treatment interactions at each time point. *RANK*, along with the *RANK* ligand, are members of the TNF family of genes that, with osteoprotegerin (OPG), form a signaling network that regulates bone mineral density [46]. Intriguingly, *RANK* ligand and OPG are expressed in adipose tissue [47], and OPG levels have been shown to be reduced by a weight loss intervention [48]. Links between change in weight and bone mass density are well described and further highlight reciprocal pathways of influence in adipose and bone [46]. *RANK* has been associated with percentage fat mass in animal linkage studies [49] and body mass index in humans [50]. Our results provide support for a novel role for the *RANK*-*RANK* ligand-OPG pathway in adipose tissue response to a lifestyle intervention in addition to their well-established role in bone.

Of the suggestive associations with year-1 weight loss, at least 2 were of particular note. The strongest suggestive association occurred within *AANAT* (rs12452844, $p = 3.12E-06$). This gene codes for arylalkylamine N-acetyltransferase, a critical enzyme in melatonin production and regulation of circadian rhythm, and has previously been associated with delayed sleep onset syndrome [51]. As epidemiologic studies document associations between short sleep and body mass index [52], this provides sug-

gestive evidence that variation in *AANAT* may contribute to that association. A suggestive association was also seen for *TBC1D1*. *TBC1D1* is an insulin-sensitive regulator of GLUT4 function in skeletal muscle, and variation in *TBC1D1* may alter glucose uptake during exercise [53]. A non-synonymous polymorphism in the *TBC1D1* gene has also been associated with severe familial obesity in at least two independent studies [54, 55].

Genetic Predictors of Year-4 Weight Change and Weight Regain

For weight change between baseline and year 4, a different pattern of results emerged. The effects on weight change occurred primarily within the DSE group, with no weight loss intervention, or when pooled across the ILI and DSE. This suggests that SNPs were often associated with naturalistic change in weight over time in DSE or that SNPs were associated with naturalistic weight change in DSE and resistance to weight loss or weight regain in ILI. It is of note, however, that *TNFRSF11A* rs17069904 continued to show evidence of association with weight change in ILI at year 4 ($p = 0.0002$) and SNP $\times$ treatment arm interaction ($p = 0.0002$).

For weight change from year 1 to 4 among those who had lost $\geq 3\%$ at year 1, SNP associations were also primarily seen in the subset of individuals randomized to DSE who had lost at least some weight at year 1. In several instances, a weaker but consistent effect in ILI resulted in a SNP effect on weight change as averaged across treatment arms. In a few instances, a weaker effect in the opposite direction occurred resulting in a suggestive interaction ($FDR\ q < 0.20$).

The causes of weight changes during the first 4 years after randomization are likely to be different between the ILI and DSE groups at different time points. The mean weight loss trajectory in the ILI group was typical of most behavioral weight loss programs, i.e. maximal weight loss at 1 year with a tendency for partial regain in the next 3 years. The initial weight loss was presumably due to intensive behavioral efforts with the regain representing a combination of inability to sustain the behavioral changes and potentially physiologic adaptations to weight loss. By contrast, the gradual, relatively steady, weight loss in the DSE group was presumably due primarily to joint effects of aging and increasing duration of diabetes which, at least in some observational studies, are associated with weight loss [56]. Therefore, one might expect the predictors (including genetic) of weight change to differ between the two treatment groups and at different time points of follow-up.

It is nonetheless notable that several of the SNP associations with year-4 weight change, either from baseline to year 4, or year 1 to 4, among those who initially lost $\geq 3\%$, occur in pathways integrally involved in structural remodeling of adipose tissue and fibrosis accumulation with increasing fat mass [57]. Proteolytic systems, such as the matrix metalloproteinase (MMP) system, contribute to tissue remodeling by degradation of the extracellular matrix and membrane components or by activation of latent growth factors [58]. MMP 13 (collagenase 3; *MMP13*) is expressed in adipose tissue with increases in expression after high-fat feeding [59]. Tissue inhibitor of metalloproteinases-3 (*TIMP3*) is 1 of 4 tissue inhibitors of metalloproteinases that have been characterized and are able to inhibit the activities of MMPs [60, 61]. MMPs release transforming growth factor- β (TGF- β), which alters cell migration and further regulates extracellular matrix and promotes fibrosis [62]. *TGFB3* is expressed in adipose tissue and downregulated in response to caloric restriction [63]. *ITGAV* encodes integrin α chain V. Integrins also interact with extracellular matrix ligands and may play a role in adipocyte apoptosis [57]. *PLA2G4F* is a member of the phospholipase family that hydrolyzes phospholipids into arachidonic acid and may play a role in fat deposition and the storage of lipids in adipose tissue [64]. The MMP and PLA_2 pathways have been shown to alter the degree of weight gain in response to high-fat diets in animal models [65–67].

Other SNP associations with weight change between baseline and year 4 were related to immune function. *IRF5* is expressed in adipose tissue and mediates proinflammatory cytokine release. A high-fat diet has been shown to upregulate *IRF5*. *FOXP1* has been associated with cytokine production of macrophages [68]. Finally, *GRB2* and *INSR* increase PI3K signaling to regulate glucose uptake. Thus, a number of the SNP associations with year-4 weight change or regain occur within plausible biological pathways of direct relevance to adipose tissue and inflammation.

Strengths and Limitations

This study has several strengths. It is the largest RCT of behavioral weight loss, with nearly 3,900 individuals with genetic consent randomly assigned to either an effective ILI focusing on weight loss and physical activity promotion or a minimal contact control group. This design presents several distinct advantages to studying gene $\times$ environment interaction relative to epidemiologic studies, including random assignment on a 1:1 basis to an environmental exposure with a large effect size. Further,

we accurately measure degree of intentional weight loss and extent of weight regain with longitudinal measures and excellent retention rates. These phenotypes are of key public health importance given the well-recognized obesity epidemic and success of behavioral weight loss in reducing cardiovascular and diabetes risk. Yet, they are inherently difficult to assess in epidemiologic studies due to difficulties with self-report and the potential for confounding by unintentional weight loss due to illness. The potential for more direct clinical application is also augmented using this design to study gene $\times$ environment interaction as the genetic effects are established in the context of a well-established and effective treatment protocol.

Importantly, we also present the first chip-wide analysis of intentional weight loss and regain and identify novel loci related to weight change phenotypes. Our genotyping platform was comprised of SNPs within over 2,100 genes related to cardiovascular disease and its risk factors. Thus, these SNPs may not be newly discovered as related to cardiometabolic phenotypes, but none had been identified previously in relation to weight change. This supports the possibility that careful genetic study of phenotypes in different environments or in response to different treatments may yield new insights into genetic architecture. It is further plausible that the inclusion of a greater number of SNPs selected with a more agnostic approach, such as GWAS or next-generation sequencing, may discover additional loci related to weight loss and regain. Finally, replication of these loci in an independent sample would further strengthen the interpretation of these results. We presented suggestive associations in addition to those reaching chip-wide significance to support these efforts. Nonetheless, it is important to note that this cohort was selected for type 2 diabetes and overweight, which may complicate replication efforts.

Conclusions

Overall, this largest chip-wide study of genetic predictors of weight loss and weight regain identifies SNPs within *ABCB11* and *TNFRSF11A* as predictors of the magnitude of weight loss during a behavioral weight loss intervention. These genetic associations highlight the potential role of bile salt transport and the TNF superfamily as novel mechanisms contributing success in weight loss with behavioral efforts. Furthermore, these results indicate that discovery efforts for genetic predictors of novel phenotypes, such as weight loss and weight regain, may yield new insights into the genetics of obesity and treatment response.

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