A common variant in the CLDN7/ELP5 locus predicts adiponectin change with lifestyle intervention and improved fitness in obese individuals with diabetes

L. Maria Belalcazar,1 George D. Papadonatos,2 Jeanne M. McCaffery,3 Inga Peter,4 Nicholas M. Pajewski,5 Bahar Erar,2 Nicholette D. Allred,6 Ashok Balasubramanyam,7 Donald W. Bowden,6 Ariel Brautbar,2 F. Xavier Pi-Sunyer,8 Christie M. Ballantyne,7,9 Gordon S. Huggins,10 and the Look AHEAD Research Group

1Department of Medicine, University of Texas Medical Branch, Galveston, Texas; 2Department of Biostatistics, Brown University, Providence, Rhode Island; 3Weight Control and Diabetes Research Center, Department of Psychiatry and Human Behavior, The Miriam Hospital and Brown Medical School, Providence, Rhode Island; 4Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, New York; 5Department of Biostatistical Sciences, Wake Forest University Health Sciences, Winston-Salem, North Carolina; 6Department of Biochemistry and Center for Genomics and Personalized Medicine Research, Wake Forest University Health Sciences, Winston-Salem, North Carolina; 7Department of Medicine, Baylor College of Medicine, Houston, Texas; 8Department of Medicine, Columbia University, St. Luke’s-Roosevelt Hospital, New York, New York; 9Center for Cardiovascular Disease Prevention, Methodist De Bakey Heart and Vascular Center, Houston, Texas; and 10MCRI Center for Translational Genomics, Tufts Medical Center, Boston, Massachusetts

Submitted 23 October 2014; accepted in final form 10 March 2015

Belalcazar LM, Papadonatos GD, McCaffery JM, Peter I, Pajewski NM, Erar B, Allred ND, Balasubramanyam A, Bowden DW, Brautbar A, Pi-Sunyer FX, Ballantyne CM, Huggins GS, Look AHEAD Research Group. A common variant in the CLDN7/ELP5 locus predicts adiponectin change with lifestyle intervention and improved fitness in obese individuals with diabetes. Physiol Genomics 47: 215–224, 2015. First published March 10, 2015; doi:10.1152/physiolgenomics.00109.2014.—Overweight/obese individuals with Type 2 diabetes have low adiponectin levels, which may improve with lifestyle changes. We investigated whether genetic variants associated with adiponectin levels in genome-wide association studies (GWAS) would also be related with adiponectin changes in response to an intensive lifestyle intervention (ILI), potentially through mechanisms altering the adipose microenvironment via weight loss and/or improved cardiopulmonary fitness. Look AHEAD was a randomized trial comparing the cardiovascular benefits of ILI-induced weight loss and physical activity compared with diabetes support and education among overweight/obese individuals with Type 2 diabetes. In a subsample of Look AHEAD with adiponectin data and genetic consent (n = 1,351), we evaluated the effects of 24 genetic variants, demonstrated by GWAS to be cross-sectionally associated with adiponectin, on adiponectin change 1-yr postintervention. We explored via mediational analyses whether any differential effects by treatment arm were occurring through weight loss and/or improved fitness. A variant, rs222857, in the CLDN7 locus, potentially associated with epithelial barrier integrity and tight junction physiology, and a putative cis expression quantitative trait locus for elongator acetyltransferase complex subunit 5 (ELP5), predicted adiponectin increases within ILI (log-adiponectin in overall sample per copy: β ± SE = 0.05 ± 0.02, P = 0.008; in non-Hispanic whites: 0.06 ± 0.02, P = 0.009). The favorable effects of rs222857 (minor allele frequency 45.5%) appeared to be mediated by mechanisms associated with improved fitness, and not weight loss. This is the first study to identify a genetic variant that modifies adiponectin response to lifestyle intervention in overweight/obese diabetic individuals.

Address for reprint requests and other correspondence: L. M. Belalcazar, Dept. of Medicine, Div. of Endocrinology and Metabolism, Univ. of Texas Medical Branch, Galveston, TX 77555-1060 (e-mail: lmbelalc@utmb.edu).

ADIPONECTIN, AN ADIPOSE TISSUE hormone with insulin-sensitizing and anti-inflammatory effects, is decreased in obesity and Type 2 diabetes (T2DM) (56). However, circulating levels of this major adipose tissue hormone do not consistently relate to the extent of adiposity and may be normal in individuals known to have a metabolically healthy obese phenotype (1). Factors that stress the adipocyte, such as an increased intake of saturated fat (47) and chronic subclinical endotoxemia, known to play a role in the development of metabolic disease (4, 5, 11, 36), contribute to the development of adipose tissue dysfunction and a reduction in adiponectin levels. Lifestyle interventions that target weight loss have been demonstrated to improve adiponectin levels in some (3, 16), but not in all studies (8, 42). These disparate responses could partly be the result of biological determinants of adipose function under the influence of genetics.

Genome-wide association studies (GWAS) have explored the effects of genetics on adiponectin and its metabolic phenotypes. Variants in the adiponectin gene (ADIPQO) have been identified as being associated with adiponectin levels, yet contributing modestly to the variance in circulating hormone levels (23, 41). Furthermore, the relationship of ADIPOQ to glucose and/or adiposity phenotypes has been inconsistent (20, 41, 46, 55). On the other hand, variants in genes outside ADIPOQ that encode proteins that impact adipose tissue function have been consistently associated with cross-sectional adiponectin levels and related metabolic phenotypes. This is true of variants that modify the ability of adipose tissue depots to differentiate and expand, e.g., those near the IRS1 (26), or those in CDH-13, which codes for T-Cadherin (9), an adiponectin receptor that mediates adiponectin’s proangiogenic effects. However, little is known about how these or other
genes control adiponectin change. We hypothesized that single nucleotide polymorphisms (SNPs) associated with adiponectin levels in cross-sectional GWAS may predict favorable changes in adiponectin levels in response to lifestyle intervention if also implicated in pathways known to favorably alter the adipose tissue microenvironment. To this effect we examined adiponectin and genetic data from overweight/obese subjects with T2DM who participated in the Look AHEAD (Action for Health In Diabetes) study (45), a cardiovascular outcome study that randomized participants to an intensive lifestyle intervention for weight loss (ILI) or to diabetes support and education (DSE). We also performed exploratory analyses to examine the potential mediational effects of weight loss and increased cardiorespiratory fitness on the association of the genetic variants with adiponectin change.

**MATERIALS AND METHODS**

**Study design.** The Look AHEAD study design, intervention, and participant characteristics have already been described (45), as have changes in weight, fitness, and major metabolic phenotypes at 1 yr, when the intervention was most intense (35). In brief, 5,145 ethnically diverse overweight/obese subjects with T2DM (and aged 45–76 yr) from 16 clinical centers were randomized either to ILI, aiming for a 7% weight loss from baseline, or to a control DSE arm. ILI participants attended three group sessions and one individual monthly encounter during the initial 6 mo, followed by two group sessions and one individual monthly encounter thereafter, in support of behavioral change to lose weight by increasing physical activity to 175 weekly minutes of moderate-intensity exercise and reducing caloric intake. The activity program relied on at-home exercise, mostly brisk walking. The energy intake goal was 1,200–1,500 kcal/day if body weight was <114 kg, and 1,300–1,800 kcal/day if weight was ≥114 kg. DSE participants received three group health information sessions during the year. All participants continued care with their primary providers. The institutional review boards of the participating centers approved Look AHEAD and this ancillary study.

Among the 5,145 participants in Look AHEAD, 4,322 participants provided consent for genetic analyses. 4,047 contributing DNA data that passed quality control procedures; 2,360 first- or second-degree relatives at 15 study sites consented for biomarker measurements. This study evaluated the 1,351 Look AHEAD participants who had quality genetic data, and adiponectin levels at baseline and year 1. We determined whether genetic variants previously showing cross-sectional association with adiponectin would also be associated with 1 yr change in adiponectin levels in response to the intervention and whether the association of the genetic variants with adiponectin change could potentially be mediated by mechanisms related to weight loss and/or improved cardiovascular fitness.

**Biomarker assays, anthropometry, and fitness determinations.** Plasma adiponectin levels were measured in duplicate in fasting plasma samples with a sandwich ELISA (American Laboratory Products, Salem, NH), as previously reported (3). Sensitivity of the assay was 0.019 ng/mL. Average intra- and interassay coefficients of variation were 2.3 and 9.6%, respectively. Procedures for anthropometric measures, including body mass index (BMI), have been reported (35). Cardiorespiratory fitness was defined as the estimated level of metabolic equivalents of task (METS; 1 MET = 3.5 ml·kg⁻¹·min⁻¹ of oxygen uptake) achieved on a treadmill work load (speed and grade) at 80% of maximal heart rate (submaximal), or at a rating of 16 on the rating of perceived exertion (RPE) scale for participants on beta-blockers, as described previously (25). The grade of the treadmill was initially set at 0% and increased by 1% at 1 min intervals; speed was set at a level between 1.5 and 4.0 mi/h, based on participant preference and heart rate response during the first minute of the test. Heart rate was assessed at rest, during the last 10 s of each exercise stage, and at the point of test termination by a 12-lead electrocardiogram. RPE was assessed according to the Borg 15-category scale (range is on a scale from 6 to 20) during the last 15 s of each stage and at the point of test termination.

**Genotyping and SNP selection.** Genomic DNA extraction was carried out with the FlexiGene DNA Kit (Qiagen, Valencia, CA) as described by the manufacturer. DNA quantitation was performed using the PicoGreen dsDNA Quantitation Reagent (Invitrogen, Carlsbad, CA). Genotyping was carried out with the Metabochip, a custom 200,000 SNP platform designed based on the GWAS meta-analyses of 23 traits related to T2DM, coronary artery disease, and myocardial infarction (53). SNPs found to be associated with adiponectin levels by GWAS were identified by searching in April 2013 the term “adiponectin” in the HUGE Navigator and then confirmed by manual PubMed Search. SNPs reported to be associated with adiponectin levels were cross-referenced with the content of the Metabochip assay. If the GWAS SNP was not available in the Metabochip, a surrogate SNP (r² > 0.8 with GWAS SNP) was sought in at least one of the race/ethnic groups represented in Look AHEAD. To ensure quality control, subjects with failed genotyping, sex inconsistency, high degree of discordance with prior genotyping, and familial relatedness were excluded. SNPs with genotyping call rate <95% and marked deviation from Hardy-Weinberg equilibrium (P < 10⁻⁶) in any race/ethnic group were also excluded. Final genetic analyses were based upon 24 SNPs (rs2494195, rs4846567, rs2943656, rs2673141, rs1083798, rs1108842, rs4301033, rs864265, rs6773957, rs6450176, rs4311394, rs1358980, rs668459, rs592423, rs2954030, rs10885531, rs7938266, rs2657888, rs245722, rs2952799, rs13332623, rs222857, rs731839, and rs8182584), all with minor allele frequency in excess of 10% and residual genotyping success rate of 99.98%.

**Statistical analysis.** Four primary ancestral population groups were distinguished via self-report of race and ethnicity: non-Hispanic whites (NHW, 72%), African American (12%), Hispanic (9.5%), and Native American (3.3%). Given the inclusion of a multiethnic and multiracial sample in Look AHEAD that attempted to mirror the U.S. population, we opted to not exclude participants based on their race/ethnicity, but to use principal component analysis of genetically derived ancestry to correct the associations of interest for hidden population stratification and admixture. In these analyses, self-reported ancestral ethnic identity was replaced by information obtained on a chipwide basis from ancestry-informative markers, after excluding rare SNPs and those in linkage disequilibrium (LD, r² > 0.30). In particular, EIGENSTRAT was used to compute principal components (PCs) for use as covariates in both the overall sample and in NHW-specific analyses, as individuals of European ancestry are not considered to be perfectly genetically homogeneous (24).

Analyses were performed in the overall sample and separately for NHW. Using the method of Li and Ji (32), we computed a multiplicity-adjusted threshold for significance as P ≤ 0.0023, taking into account the effective number of 22 independent hypotheses tested among 24 SNPs selected for having previously established associations with adiponectin. Given the exploratory nature of our study, associations at a level of P < 0.05 were considered as nominal and further evaluated.

Adiponectin values were log-transformed prior to analysis to correct for nonnormal distribution. Baseline and 1 yr measurements were modeled jointly with an unstructured covariance matrix. Three-way interaction models of individual SNP markers (0, 1, or 2 copies of the minor allele; additive model) with measurement time (1 yr vs. baseline) and study arm (ILI vs. DSE) were estimated in S-Plus 8.2 (Tibco Software, 2010) using restricted maximum likelihood. Three distinct types of SNP effects are presented, which can be interpreted as the effect of one additional copy of the corresponding minor allele on: 1) baseline adiponectin levels for both treatment arms combined, 2) 1 yr change in adiponectin levels within each of the two treatment arms (ILI and DSE), and 3) ILI vs. DSE differences in 1 yr change in adiponectin (SNP × time × treatment interaction). Regression models
also adjusted for age at interview, sex; genetic ancestry (top three PCs: pca1, pca2, and pca3), clinic site; BMI, fitness, baseline HDL-cholesterol (HDL-C), baseline log of triglycerides, baseline hemoglobin A1c (HbA1c), smoking; and use of insulin, thiazolidinediones (TZDs), and statins at each visit (baseline and year 1). All of these covariates, other than site, were allowed to have time- and study arm-specific effects (covariate $\times$ time $\times$ treatment interactions).

To explore whether change in adiposity (BMI) or change in fitness (METs) mediated the effects of the identified genetic variant(s) on response to the intervention, we used the approach of Baron and Kenny (2). In particular, we compared regression models for adiponectin change that controlled only for baseline values of BMI and fitness, with models that additionally controlled for changes in BMI and in fitness. In conjunction with multivariate regression models for BMI and METs of the same form as those fitted for adiponectin (see above), we were able to obtain parameter estimates and $P$ values for the effect of the identified SNP(s) on mediators (BMI change and METs change), effect of mediators on adiponectin change, and SNP effects on adiponectin change with and without mediator effects. Significance of indirect intervention effects was estimated via Sobel’s test (49). The variance inflation factor (VIF), due to correlation between the two putative mediators, was well below levels indicative of collinearity ($VIF < 1.3$) (7), allowing us to evaluate them jointly in a multiple mediation setting (39). Furthermore, changes in BMI captured changes in weight in this adult-only sample. We did not examine change in waist circumference, given that previous work in Look AHEAD (Belalcazar LM, Lang W, Haffner SM, Schwenke DC, Kriska A, Balasubramanyam A, Hoogeveen RC, Pi-Sunyer FX, Tracy RP, Ballantyne CM; unpublished) showed that change in waist accounted for a slightly smaller or a similar proportion of the variance in adiponectin change in obese men and women with diabetes than in change in weight.

## Results

### Baseline characteristics and 1 yr changes.

The 1,351 participants in this Look AHEAD biomarker-genetics cohort had a mean age of 58 yr; 56% were women and 72% were NHW (Table 1). Participants were obese (average BMI of 36.5 kg/m$^2$), and glucose control was slightly above target (mean HbA1c of 7.3%). Characteristics of participants in the ILI arm at baseline ($n = 688$) did not differ from DSE participants ($n = 663$). Adiponectin levels were low in Look AHEAD participants compared with those in individuals without TZDM, as previously described (3), with higher levels in women than in men. Sample characteristics of the adiponectin and genetic subsets have already been reported (3, 15). Participants with adiponectin data were slightly younger and had a slightly lower prevalence of cardiovascular disease than did the remainder of Look AHEAD participants (3) likely because Look AHEAD modified its age eligibility criteria during the second year of the study, resulting in a slightly younger age for our participants. In addition, our subset had a lower representation of African Americans than did the overall cohort, given the lower rate of consent for genetic testing among participants in this racial category (15).

Similar to the full Look AHEAD cohort (35), ILI participants in this substudy showed significant weight loss at 1 yr and improvements in fitness, glucose control (HbA1c), HDL-C, and in triglycerides. ILI participants also benefited from a reduction in the use of insulin, TZDs, and statins compared with those in the DSE arm (Table 2). Adiponectin levels increased significantly with ILI compared with DSE and

### Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall ($n = 1,351$)</th>
<th>ILI ($n = 688$)</th>
<th>DSE ($n = 663$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr; mean (SD)</td>
<td>57.6 (7.2)</td>
<td>57.6 (7.3)</td>
<td>57.7 (7.2)</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>756 (56)</td>
<td>389 (56.5)</td>
<td>367 (55.4)</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>973 (72)</td>
<td>504 (73.3)</td>
<td>469 (70.7)</td>
</tr>
<tr>
<td>African American</td>
<td>158 (11.7)</td>
<td>81 (11.8)</td>
<td>77 (11.6)</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>128 (9.5)</td>
<td>61 (8.9)</td>
<td>67 (10.1)</td>
</tr>
<tr>
<td>American Indian/Alaskan Native</td>
<td>45 (3.3)</td>
<td>18 (2.6)</td>
<td>27 (4.1)</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>9 (0.7)</td>
<td>6 (0.9)</td>
<td>3 (0.5)</td>
</tr>
<tr>
<td>Other</td>
<td>38 (2.8)</td>
<td>18 (2.6)</td>
<td>20 (3.0)</td>
</tr>
<tr>
<td>CVD history, n (%)</td>
<td>170 (12.6)</td>
<td>88 (12.8)</td>
<td>82 (12.4)</td>
</tr>
<tr>
<td>Diabetes duration, yr; median (IQR)</td>
<td>5 (2 to 10)</td>
<td>5 (2 to 10)</td>
<td>5 (2 to 10)</td>
</tr>
<tr>
<td>Tobacco use, n (%)</td>
<td>44 (3.3)</td>
<td>24 (3.5)</td>
<td>20 (3.0)</td>
</tr>
<tr>
<td>Adiponectin, µg/ml; median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>4.7 (3.5 to 6.9)</td>
<td>4.7 (3.4 to 6.7)</td>
<td>4.8 (3.6 to 7.2)</td>
</tr>
<tr>
<td>Men</td>
<td>4.4 (3.1 to 6.3)</td>
<td>4.2 (3.1 to 6.0)</td>
<td>4.4 (3.2 to 6.5)</td>
</tr>
<tr>
<td>Women</td>
<td>5.1 (3.8 to 7.6)</td>
<td>5.0 (3.7 to 7.3)</td>
<td>5.3 (3.8 to 8.0)</td>
</tr>
<tr>
<td>BMI, kg/m$^2$; mean (SD)</td>
<td>36.5 (6.3)</td>
<td>36.6 (6.5)</td>
<td>36.4 (6.0)</td>
</tr>
<tr>
<td>Weight, kg; mean (SD)</td>
<td>103 (20.0)</td>
<td>104 (20.6)</td>
<td>103 (19.2)</td>
</tr>
<tr>
<td>Waist circumference, cm; mean (SD)</td>
<td>115.5 (14.7)</td>
<td>115.6 (15.0)</td>
<td>115.5 (14.3)</td>
</tr>
<tr>
<td>Fitness, submaximal, METS; mean (SD)</td>
<td>5.15 (1.47)</td>
<td>5.13 (1.44)</td>
<td>5.16 (1.50)</td>
</tr>
<tr>
<td>HbA1c, %; mean (SD)</td>
<td>7.3 (1.16)</td>
<td>7.3 (1.15)</td>
<td>7.3 (1.17)</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dl; mean (SD)</td>
<td>42.3 (11.5)</td>
<td>42.3 (11.2)</td>
<td>42.3 (11.7)</td>
</tr>
<tr>
<td>Triglycerides, mg/dl; median (IQR)</td>
<td>153 (108–225)</td>
<td>155 (110–230)</td>
<td>149 (107–222)</td>
</tr>
<tr>
<td>Medication use, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>203 (15.0)</td>
<td>100 (14.5)</td>
<td>103 (15.5)</td>
</tr>
<tr>
<td>Statin</td>
<td>560 (41.5)</td>
<td>293 (42.6)</td>
<td>267 (40.3)</td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td>376 (27.8)</td>
<td>180 (26.2)</td>
<td>196 (29.6)</td>
</tr>
</tbody>
</table>

ILI, intensive lifestyle intervention; DSE, diabetes support and education; CVD, cardiovascular disease; METS, metabolic equivalents of task; IQR, interquartile range; BMI, body mass index.

Physiol Genomics • doi:10.1152/physiolgenomics.00109.2014 • www.physiolgenomics.org
more so in male than in female participants, in agreement with our previous report from Look AHEAD (3).

### Genetic determinants of adiponectin levels at baseline.

Among the 24 SNPs found to be associated with adiponectin levels by GWAS and that met eligibility criteria for our study, ADIPOQ variant rs6773957 was found to be significantly associated with baseline adiponectin levels (logarithmic scale), in both the overall group ($\beta \pm SE = 0.06 \pm 0.02, P < 0.001$) and among NHW ($\beta \pm SE = 0.08 \pm 0.02, P < 0.001$) (Table 3). In the original scale of the data ($\mu g/ml$), this corresponds to a 7% [95% confidence interval (CI): 3–10%] increase in baseline adiponectin levels per copy of the minor allele A in the overall sample, and an 8% (95% CI: 4–25%) increase among NHW. Among the remaining SNPs, previously associated with adiponectin levels in larger samples comprising mostly nondiabetic subjects, several were found to be associated with baseline adiponectin levels in our diabetes sample at nominal levels of significance, in both the overall group and in NHW, including PEPD rs731839 and CMIP rs2925979.

**Table 2. Variables of interest at 1 yr and their changes from baseline**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ILI (n = 688)</th>
<th>DSE (n = 663)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin, $\mu g/ml$; median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>5.2 (3.9 to 7.7)</td>
<td>4.8 (3.4 to 7.4)</td>
<td>0.009</td>
</tr>
<tr>
<td>Men</td>
<td>5.1 (3.7 to 7.4)</td>
<td>4.5 (3.2 to 6.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>Women</td>
<td>5.5 (3.9 to 8.0)</td>
<td>5.4 (3.6 to 7.8)</td>
<td>0.440</td>
</tr>
<tr>
<td>Absolute change from baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.6 (−0.3 to 1.7)</td>
<td>−0.1 (−0.9 to 0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men</td>
<td>0.9 (−0.1 to 1.9)</td>
<td>0.0 (−0.8 to 0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td>0.3 (−0.5 to 1.5)</td>
<td>−0.1 (−1.1 to 0.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% change from baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>13.1 (−7.0 to 37.7)</td>
<td>−1.7 (−17.6 to 18.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men</td>
<td>24.2 (−2.1 to 45.2)</td>
<td>0.8 (−16.5 to 19.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td>6.9 (−9.6 to 29.2)</td>
<td>−3.1 (−18.9 to 17.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²; mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>33.57 (6.48)</td>
<td>36.16 (6.19)</td>
<td></td>
</tr>
<tr>
<td>Absolute change from baseline</td>
<td>−3.07 (2.64)</td>
<td>−0.22 (1.86)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% change from baseline</td>
<td>−8.37 (6.75)</td>
<td>−0.56 (4.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fitness, submaximal, METS; mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>6.15 (1.91)</td>
<td>5.41 (1.64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Absolute change from baseline</td>
<td>1.03 (1.37)</td>
<td>0.25 (1.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% change from baseline</td>
<td>22.06 (29.8)</td>
<td>6.36 (21.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c, %; mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>6.6 (1.03)</td>
<td>7.1 (1.13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Absolute change from baseline</td>
<td>−0.73 (0.96)</td>
<td>−0.21 (0.90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% change from baseline</td>
<td>−9.28 (11.1)</td>
<td>−2.18 (11.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dl; mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>45.9 (12.1)</td>
<td>43.4 (12.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Absolute change from baseline</td>
<td>3.52 (6.87)</td>
<td>1.25 (6.51)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% change from baseline</td>
<td>9.7 (17.4)</td>
<td>3.9 (14.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mg/dl; median (IQR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>131 (93 to 185)</td>
<td>147 (100 to 204)</td>
<td>0.001</td>
</tr>
<tr>
<td>Absolute change from baseline</td>
<td>−21 (−69 to 14)</td>
<td>−5 (−43.5 to 27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% change from baseline</td>
<td>−15 (−36.7 to 11.2)</td>
<td>−4 (−27.6 to 20.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medication use, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>90 (13.1)</td>
<td>116 (17.5)</td>
<td>0.025</td>
</tr>
<tr>
<td>Difference from baseline†</td>
<td>−10 (−1.4)</td>
<td>13 (2.0)</td>
<td>0.005</td>
</tr>
<tr>
<td>Statins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>303 (44.0)</td>
<td>305 (46.0)</td>
<td>0.495</td>
</tr>
<tr>
<td>Difference from baseline†</td>
<td>10 (1.4)</td>
<td>38 (5.7)</td>
<td>0.055</td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>165 (24.0)</td>
<td>202 (30.5)</td>
<td>0.008</td>
</tr>
<tr>
<td>Difference from baseline†</td>
<td>−15 (−2.2)</td>
<td>6 (0.9)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*P values calculated by Student-t-test if mean (SD) reported; $\chi^2$ test if n (%) reported; and Wilcoxon rank-sum test if median (IQR) reported. †Percent change calculated as % at follow-up minus % at baseline.
To illustrate the genotypic effect of rs222857 on adiponectin change, we calculated the expected adiponectin treatment response in male and female participants, based on the overall sample, further assuming a participant age of 60 yr, a negative history of current smoking at baseline, and taking no insulin, TZDs, or statin medications at either baseline or follow-up. Based on our longitudinal statistical model and in response to ILI, male participants carrying the TT genotype showed a 14, 21, and 27% increase in adiponectin levels in the TT, TC, and CC genotypic group, respectively; female ILI participants a 4, 9, and 15% increase in adiponectin levels, in the TT, TC, CC genotypic group, respectively, with a rate of increase that was 5% higher per C allele copy in both men and women (Fig. 1).

Two other genetic variants were associated with adiponectin change in the overall sample, albeit with only suggestive evidence of an interaction with treatment arm ($P < 0.10$, Table 3).

Table 3. Genetic determinants of adiponectin levels at baseline and of 1 yr changes in adiponectin by treatment arm

<table>
<thead>
<tr>
<th>Genetic Variant Characteristics</th>
<th>Sample</th>
<th>ILI and DSE ($n = 1,351$ overall; $n = 973$ NHW)</th>
<th>1 yr Change*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP Major/Minor Allele</td>
<td>MAIF</td>
<td>Group</td>
<td>B</td>
</tr>
<tr>
<td>LYPLAL1 rs2494195 G/A</td>
<td></td>
<td>overall</td>
<td>0.03</td>
</tr>
<tr>
<td>NHW rs4846567 G/T</td>
<td>36.13</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>ADIPOQ rs6773957 G/A</td>
<td>42.52</td>
<td>0.06</td>
<td>0.02</td>
</tr>
<tr>
<td>NHW rs2925979 G/A</td>
<td>39.88</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>CMIP rs222857 T/C</td>
<td>42.14</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>NHW rs222857 T/C</td>
<td>39.70</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>CLDN7 rs731839 T/C</td>
<td>35.05</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>PEPD rs8182584 G/T</td>
<td>36.16</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>NHW rs8182584 G/T</td>
<td>40.93</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>CLDN7 rs731839 T/C</td>
<td>40.49</td>
<td>0.05</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Adiponectin was analyzed in the logarithmic scale. Regression models adjust for: age at interview; sex; ancestry (principal components 1–3); clinic site; baseline BMI, baseline fitness, baseline HDL-C, baseline log of triglycerides, baseline HbA1c, and smoking; and use of insulin, thiazolidinediones and statins at each interview time. Tx, treatment; SNP, single nucleotide polymorphism; MA, minor allele; MAF, minor allele frequency; NHW, non-Hispanic whites; LYPLAL1, lysophospholipase-like 1; ADIPOQ, adiponectin; CMIP, c-Maf inducing protein; CLDN7, claudin-7; PEPD, prolidase/peptidase D.
3): *LYPLAL1* rs4846567 was nominally associated with adiponectin change within the ILI arm (β ± SE = 0.06 ± 0.02, *P* = 0.007), but not in the DSE arm (β ± SE = 0.00 ± 0.02, *P* = 0.95). Furthermore, *PEPD* rs8182584 was nominally associated with adiponectin change in the DSE arm (β ± SE = 0.05 ± 0.02, *P* = 0.026), but not in the ILI arm (β ± SE = 0.00 ± 0.02, *P* = 0.89). *ADIPOQ* variant rs6773957, which was strongly associated with baseline adiponectin levels, had no association with adiponectin response to lifestyle intervention (*P* > 0.10, Table 3) and did not demonstrate a differential response across study arms (interactions with treatment arm were *P* = 0.27 and 0.11 in the overall and NHW groups, respectively).

Changes in fitness and fatness as mediators of rs222857 effects on adiponectin response to lifestyle intervention. Given the novel suggestive association of rs222857 with adiponectin change in response to ILI (*P* = 0.008), it was of interest to determine whether this change was potentially mediated by change in adiposity [BMI change (Δ)] and/or change in fitness (METs Δ). To this effect, analyses for adiponectin change within the ILI arm were repeated in the overall sample (*n* = 1,351) and in NHW (*n* = 973) with additional covariate adjustment for BMI Δ and METs Δ (collinearity between the two putative mediators was low: VIF = 1.25 in the overall sample and 1.28 in NHW). Because of missing fitness data at 1 yr follow-up, 134 participants did not contribute to ILI change estimates. Values shown in Fig. 2 are regression parameter estimates (standard errors) and *P* values for the following paths: a1 and a2, SNP effects on the putative mediators; b1 and b2, effects of mediators on adiponectin change; c and c’, SNP effects on adiponectin change estimated without, and with, change in mediators, respectively. Indirect intervention effects (a1b1 and a2b2) were significant for METs Δ (Sobel’s Z = 2.293, *P* = 0.022), but not for BMI Δ (Sobel’s Z = 0.571, *P* = 0.568). In NHW alone the indirect intervention effects (a1b1 and a2b2) were again found to be significant for METs Δ (Sobel’s Z = 2.221, *P* = 0.026), but not for BMI Δ (Sobel’s Z = 0.423, *P* = 0.672). These results suggest that change in fitness (METs Δ), and not fatness (BMI Δ), may be activating the mechanisms that link rs222857 with improvements in adiponectin levels in response to ILI (Fig. 2).

**DISCUSSION**

There are two main genetic findings from this study that relate to our original hypothesis. The first suggests that while SNPs in the *ADIPOQ* gene are predictive of baseline adiponectin levels in the presence of diabetes, they are not related to changes in adiponectin levels in response to lifestyle intervention. We found that the *ADIPOQ* variant rs6773957, which showed the strongest association with baseline adiponectin levels, had no association with response to lifestyle intervention and did not demonstrate a differential response across study arms. A similar observation was made in nonobese individuals examined in The Finnish Diabetes Prevention study (*n* = 190) (48). Of note, rs6773957 is in complete LD with *ADIPOQ* rs3774261, which has been demonstrated by Mendelian randomization studies to be associated with adiponectin levels, as well as with insulin sensitivity (20). The positive Mendelian randomization finding, our results, and those of the Finnish study suggest that the association of *ADIPOQ* rs6773957 with adiponectin levels may be consistent through life, despite individual differences in behaviors related to moderate changes in weight and fitness, and may not be substantially modifiable by lifestyle interventions for weight loss.

Our second major finding lends support to the hypothesis that variants of genes implicated in pathways that alter the adipose tissue microenvironment may be associated with increases in adiponectin levels in response to lifestyle intervention. We identified rs222857, a SNP in the *CLDN7* locus, to be associated with adiponectin change in the ILI, but not in the DSE arm, with a nominally significant SNP × treatment interaction, both in the overall cohort and in NHW. SNP rs222857 was selected for analysis as part of this study because it served as a proxy for the variant rs6773957 (46.241 bp away, *r*² = 0.804 and D’ = 0.927), which has been associated with adiponectin levels (12). Variant rs6773957 is located in *DLG4*, a gene coding for postsynaptic density protein 95, which, like
CLDN7, is involved in the regulation of tight junction physiology (19).

rs222857 has been identified as an intron variant in CLDN7, a gene that codes for claudin-7, a member of a family of transmembrane proteins (37) known to be structural and functional components of tight junctions and key regulators of epithelial barrier function (22). Although claudin-7 is expressed in other organs, including kidney and lung, where it is also a regulator of epithelial permeability (22), its expression in the intestinal basolateral membrane and interaction with other membrane proteins to maintain cell-matrix and intestinal epithelium integrity (18) may affect adipose tissue, where adiponectin is synthesized and secreted. Claudin-7-deficient mice demonstrate sloughing of the intestinal epithelium and activation of an acute inflammatory response (14). A “leaky gut” is present in individuals with diabetes and obesity (11, 30). In the setting of increased intestinal permeability, bacteria and bacterial components, including lipopolysaccharide (LPS), cross the intestinal barrier (5) and bind to Toll receptor-4 (TLR4), causing inflammation and insulin resistance in liver, muscle, and adipose tissue (4, 5, 11). We speculate that the favorable effects of a CLDN-7 variant on adiponectin change with ILI could potentially be the result of a decrease in LPS leakage from the gut with a reduction in TLR4 signaling in muscle. Reduced TLR4 signaling in muscle improves insulin action, necessary for the activation of protective heat shock protein responses with exercise and their favorable effects on adipose tissue function (10, 13, 28).

Independent studies in lymphoblastoid cell lines (33, 50) have identified rs222857 as a putative cis expression quantitative trait loci (eQTL) for the elongator acetyltransferase complex subunit 5 gene (ELP5), also known as DERP6 or C17orf81 (http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/). Importantly, rs222857’s proxy, rs507506, identified by GWAS to be associated with adiponectin (12), has also been reported as a cis eQTL for ELP5 (50). ELP5 is part of Elongator, a highly conserved multiprotein complex with acetylating activity that plays an important role in transcriptional and translational regulation, mainly through histone acetylation and mRNA modification (21, 54). Elongator indirectly affects multiple cellular functions that result from its transcriptional/translational activities including DNA damage response, telomeric gene silencing, exocytosis, protein acetylation, and paternal genome demethylation (21, 34, 54). It is of interest that both SNPs, rs222857 and its proxy rs507506, have been associated with tight junction physiology and identified as putative cis eQTL for ELP5. Although transcriptional or translational modifications of CLDN7 by ELP5 have not been described, acetylation has been reported to play a role in modification of claudin expression and intestinal epithelium integrity (52). ELP5 is ubiquitously expressed in human tissues and may have effects that are independent of CLDN7. ELP5 is involved in p53-dependent transcriptional regulation (57). p53 plays an important role in metabolism, participating as a sensor of energy availability within the cell, its effects modified by acetylation (51). When cells are subject to an energy deficit, p53 activates sirtuin enzyme SIRT1 (40), a NAD+-dependent deacetylase that has been associated with longevity and that participates in the activation of cell signaling cascades that improve insulin sensitivity and glucose homeostasis (6). This finding is relevant because long-term lifestyle changes including exercise and/or regular endurance training have been associated with an increase in sirtuin activity in muscle (29) and adipose tissue (17). Downstream effectors of sirtuin activity increase adiponectin gene transcription in adipocytes and may do so independently of changes in weight (38, 43).

Our findings may offer potential mechanisms to the observations in small intervention studies suggesting that exercise training may increase adiponectin levels independently of weight loss and of adiponectin gene polymorphisms (31) and are in agreement with a growing body of literature showing that adipose tissue function (of which adiponectin is a marker) may be independent of adiposity (1) and that cardiorespiratory fitness and adiponectin levels are related (44). We acknowledge that our study has several strengths, as well as limitations. To our knowledge, we present the largest sample to date assessing the effects of genetics on adiponectin change and identify a novel locus related to this important metabolic phenotype. Although our sample size is still small compared with cohorts used for GWAS, which reduced our power to replicate all previously identified SNP associations with adiponectin levels at baseline, the present study is uniquely poised to answer questions related to intervention, an approach not amenable to these larger studies. The randomized lifestyle intervention in Look AHEAD offers distinct advantages to studying gene × environment interaction in individuals with T2DM relative to observational epidemiologic studies, including random assignment on a 1:1 basis to an environmental exposure with a large effect size, providing insights on the effects of improvements in fitness and weight loss as modifiers of genetic effects. It is 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 adiponectin change. We acknowledge that the locus bounded by rs507506 and rs222857 that includes CLDN7 and ELP5 is gene-rich and that it is not possible to precisely identify the genetic mechanism within this locus that is responsible for differential adiponectin response to lifestyle. We recognize the importance of independent replication of our findings; however, currently no cohort or randomized trial of obese patients with T2DM subjected to a randomized lifestyle intervention exists. Similar intervention trials, without data on fitness changes, have been performed in smaller samples in less obese individuals who do not have diabetes (27, 48). It is unlikely that this expensive intervention trial will be replicated in a larger sample in the future. With these considerations in mind, we found it justified when designing our study to include findings that not only fulfilled the strict criteria for statistical significance imposed by Li and Ji (32), but also those that could be of potential physiological significance and that would otherwise be excluded. Therefore, a P value of <0.05 was defined as nominally significant for the identification of potential associations. In addition, we note that our results may be most reflective of NHWs and may not be generalizable to all racial and ethnic groups or to a less obese population without diabetes. Sample size limitations precluded examination of potential differences between men and women in the association of rs222857 with adiponectin change in response to lifestyle intervention.

In summary, our results bring attention to novel potential links between ELP5, an effector of transcriptional/translational regulation of an energy-sensing molecule (p53), and/or...
CLDN7, a determinant of epithelial barrier integrity, and adiponectin change. Our findings remain hypothesis-generating but propose that the association of variant rs222857 with adiponectin change in response to ILLI in a large subset of Look AHEAD participants is partly mediated by mechanisms that relate to improved cardiorespiratory fitness.

ACKNOWLEDGMENTS

For a listing of Look AHEAD Research Group Investigators and funding for the Look AHEAD Study please refer to the supplemental file.1

Trial registration: NCT0017953.
Current address for A. Brautbar: Dept. of Medical Genetics, Marshfield Clinic, Marshfield, WI.

GRANTS

This work represents a collaboration from two Look AHEAD Ancillary Studies: The Obesity, Inflammation and Thrombosis Ancillary Study and the Genetics and Ancillary Study, with funding from National Institutes of Health Grants HL-09051401 (L. M. Belalcazar, C. M. Ballantyne) and DK-090043 (G. D. Papandonatos, J. M. McCaffery, I. Peter, and G. S. Huggins).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

1. Aguilar-Salinas CA, Garcia EG, Robles L, Riano D, Ruiz-Gomez DG, Garcia-Ulloa AC, Garcia-Escalante J, Delzenne NM, Alessi MC, Burcelin R. Adiponectin change. Our findings remain hypothesis-generating but propose that the association of variant rs222857 with adiponectin change in response to ILLI in a large subset of Look AHEAD participants is partly mediated by mechanisms that relate to improved cardiorespiratory fitness.


The online version of this article contains supplemental material.

Genetics, Adiponectin Change, and Fitness

Physiol Genomics • doi:10.1152/physiolgenomics.00109.2014 • www.physiolgenomics.org


