rs4771122 predicts multiple measures of long-term weight loss after bariatric surgery

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Abstract

We examined the association of 34 single nucleotide polymorphisms with weight loss up to 9.5 years after Roux-en-Y surgery. Participants were enrollees in the NUgene biobank with stored DNA and linked electronic health records. Ninety-five self-identified white participants underwent surgery and had follow-up weights obtained between 1 and 9.5 years after surgery. SNP rs4771122 was the variant most significantly associated with long term weight loss after surgery in a repeated linear mixed model (p = .004) of long-term weight loss. In this model, each additional copy of the minor allele was associated with nearly 5 percent greater percentage weight loss. This same SNP was also nominally significantly (p < .05) associated with weight loss trajectories, weight loss nadir, and weight loss 2 years after surgery.
**INTRODUCTION**

There is wide variability in weight loss after Roux-en-Y gastric bypass (RYGB) but the reasons for this variability are incompletely understood. Single nucleotide polymorphisms (SNPs) known to be associated with obesity or waist-to-hip ratio (WHR) in genome-wide association studies (GWAS) are logical targets in investigations into genetic contributors to this variation. A few published studies have examined obesity or WHR GWAS SNPs with weight loss after bariatric surgery (1–3) but have either included only a few SNPs, used simplistic statistical methods, or used datasets with limited follow-up. To better understand how these SNPs may contribute to long-term weight loss after RYGB surgery, we examined the association of 34 SNPs with weight loss up to 9.5 years after bariatric surgery using linear mixed methods and several secondary measures of long-term weight loss.

**MATERIALS AND METHODS**

**Participants and data**

Participants for this study were drawn from the NUGene biobank; individuals in NUGene were recruited at Northwestern Medicine Clinics or Evanston Northwestern Hospital (now NorthShore University HealthSystem), provided a blood sample for DNA extraction, completed an entrance questionnaire, and gave permission for use of their electronic health records (EHR) in research. Participants in NUGene who had undergone RYGB surgery were identified through International Classification of Diseases -9-9 and Current Procedural Terminology codes. Information about pre- and post-surgical weights were extracted from EHR while demographic data, including race, were obtained from the entrance questionnaire. Details about assembly of the study population and data extraction have been published previously (4). Patients undergoing reoperations and surgical-take downs were excluded.

**Genotyping**

We sought to genotype all SNPs that reached genome-wide levels of significance in the largest (at the time of genotyping) GWAS of BMI (5) and WHR (6). The iPLEX GOLD procedure was followed for the SNP analysis. Briefly, Sequenom’s Assay Design software was used to create five multiplexed reactions for all SNPs of interest. After extending the primers the products were conditioned and spotted onto SpectroChip Arrays. Extended primers were detected by MassArray Analyzer 4 using matrix-assisted laser desorption ionization–time-of-flight (MALDITOF) method. Data was acquired using the TyperAnalyzer Software from Agena Bioscience (formerly Sequenom). During the design phase of the assay, it was determined that some SNPs would not genotype well on the system and the SNAP browser was used to pick suitable proxies in the Caucasian population. All SNPs having genotyping rates below 95% were discarded, as were SNPs...
with HWE p-values less than .01. 34 of 46 SNPs passed these quality control filters. The rs numbers of these SNPs are included in a footnote to Table 1.

Outcome Measures

In all models, percent weight lost from surgery ([post-surgical weight values - surgery weight]/surgery weight) was used as it has been suggested this phenotype facilitates the most sensitive identification of novel predictors of surgery-induced weight loss (7). We included all individuals (n=162) who had at least 1 weight observation 1-year after surgery, and subsequently restricted this to genotyped self-reported whites (n=95). Our primary outcome was long term percent weight loss predicted by a linear mixed model. We chose a linear mixed model including repeated measures of weight obtained at least 1 year after bariatric surgery with a random intercept and unstructured covariance matrix. Each SNP was tested individually in an unadjusted linear mixed model with and without an additional test for interaction with time. In recognition of limited power and to replicate earlier studies, we chose to examine multiple secondary measures of weight loss after bariatric surgery, and to prioritize those SNPs having nominally significant (p < .05) associations with multiple measures of weight loss. Our secondary outcomes included lowest percent weight loss recorded post-surgery (percent weight loss nadir), percent weight loss at 1 year (weight measure obtained closest to 1 year after surgery in a range of 12–18 months), percent weight loss at 2 years (range 18–30 months), and long term weight loss trajectories estimated by SAS Proc trajectory. We had previously used a semiparametric, group-based mixture model (SAS Proc Traj) to create trajectory models of percent weight loss from one-year post surgery in this study (4). We tested the statistical significance of each SNP against previously calculated trajectory groups using Pearson’s Chi-Squared.

After selection of independently associated demographic predictors (gender, surgery age, surgery weight, height, and recruitment center), SNPs were singly included as additional covariates modeled additively, with each additional unit reflecting an additional copy of the minor allele of each SNP. Mixed linear regression models were additionally adjusted for time since surgery and a time*age interaction term.

RESULTS

Demographics

Informed consent was obtained from all individual participants included in the study. Table 1 presents the demographics of the 95 whites with successful genotyping for analysis, both in total, and by rs477112 genotype. The median number of weight observations one-year or more after surgery per participant was 8, and the average length (SD) of follow-up after surgery was 5.6 (2.2) years, with some individuals having as long as 9.5 years of follow up. There were 1634 weight observations in the dataset. Eighty-one (85%) participants were female, reflecting a typical distribution of bariatric surgery patients in the United States.

Obesity and WHR GWAS SNP associations with long term weight loss after bariatric surgery—A table including P-values and beta estimates for the association of all 34 SNPs with our primary and secondary outcomes reflecting long-term weight loss is
available from the authors. No SNP achieved a Bonferroni corrected (.05/34 = .0017) p-value for association with long term weight loss examined in a linear mixed model. SNP rs4771122 achieved the lowest p-value for association with this trait of $p = .0035$. SNP rs4771122 was also nominally significantly associated with trajectory group membership, nadir percent weight loss, and 2 year percent weight loss. No other SNP was associated with multiple outcomes.

Table 2 presents beta estimates and p-values for the association of rs4771122 with our primary and secondary measures of long-term weight loss. In all cases, additional copies of the minor (G) allele were associated with a larger percentage weight lost, after adjustment for several variables including pre-surgical BMI. As seen in table 1, in this sample, additional copies of the minor (G) allele were also significantly associated with increased pre-surgical weight. We examined the association of rs4771122 with pre-surgical weight, nadir weight loss, 2 year weight loss, long term weight loss by mixed models, and long term weight loss by trajectories in 52 self-identified blacks genotyped for our study and found no significant associations.

CONCLUSION

In our examination of 34 obesity and WHR GWAS SNPs, SNP rs4771122 in an intron of \textit{MTIF3} was the most significantly associated SNP with long term weight loss using repeated measures of weight captured 1–9.5 years after surgery. SNP rs4772211 was also the only SNP to be associated with multiple other measures of weight loss after surgery such as percent weight loss nadir, and long term weight loss trajectory. In our sample this SNP was also nominally associated with BMI at baseline, with the minor allele of the SNP being associated with higher baseline BMI and greater weight loss after surgery. Like most GWAS discoveries to date, the mechanism of action of rs4772211 through \textit{MTIF3} (or another gene in the region) on obesity or weight loss after surgery is unknown.

Several other studies have examined genetic variation identified in obesity GWAS with weight loss after surgery, perhaps in response to one study that found individuals having rare variation in MC4R, an important obesity GWAS peak, to have different amounts of weight loss after gastric bypass (8)). Like similar studies (9), most of the GWAS SNPs examined in this study were not significantly associated with weight loss after RYGB surgery perhaps because of the relatively small effect sizes of these SNPs on obesity (paired with the limited power of this analysis in 95 individuals), or complex gene by environment interactions important in post-surgical weight loss, or because the SNPs may act through pathways other than satiety or basal metabolic rate. No other candidate gene studies examining obesity or WHR GWAS SNPs have genotyped SNP rs4771122 (1–3). Our study did not replicate previous results from some of these studies suggesting significant association between SNPs in FTO and weight loss after surgery (1, 3). Two GWAS studies have been performed examining weight loss after bariatric surgery and neither identified rs4771122 among the top hits. One GWAS study used a very different measure of long term weight loss, comparing the group with the highest excess percent body weight lost to the group with the lowest percent excess body weight lost (10). However, a second GWAS (9) used percent weight loss at weight nadir, one of our secondary variables and found SNP rs4771122 was
nominally (p = .02) associated with nadir weight loss in the replications sample, but not in the discovery sample. In the replication sample, each copy of the G allele was associated with 2.14 greater percent weight lost at the nadir (compared to 4.72 greater percent weight lost at the nadir in our study) (9). The nominal association of rs4771122 G allele with greater BMI at baseline aligns with the Speilotes et al. (5) obesity GWAS where the G allele was associated with higher BMI, but is dissimilar to a Finnish study of nearly 1000 bariatric surgery patents that did not find rs4771122 and several other obesity GWAS SNPs to be associated with BMI in patients considering bariatric surgery (11).

Strengths of this study include typing of many BMI and WHR SNPs, extended follow up with many weight measures per individual after surgery, the use of advanced statistical techniques to examine associations with weight loss, and the examination of multiple endpoints. Weaknesses include low power, lack of genome-wide genotyping, and lack of independence between multiple examined endpoints. Based on the results of this study, rs4771122 should be included in future, larger examinations of weight loss after bariatric surgery, and, where possible, these studies should be encouraged to incorporate multiple measures of long-term weight loss utilizing linear mixed methods.

Acknowledgments

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References


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<table>
<thead>
<tr>
<th>Variable</th>
<th>White Patients (n=95)</th>
<th>Rs4771122 AA (n=65)</th>
<th>Rs4771122 AG (n=26)</th>
<th>Rs4771122 GG (n=4)</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Number of Observations per Person, median(IQR)</td>
<td>8 (3–22)</td>
<td>7 (3–20)</td>
<td>16 (6–37)</td>
<td>5 (3–8)</td>
<td>0.0808</td>
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<tr>
<td>Years of Follow Up</td>
<td>5.4 (2.2)</td>
<td>5.2 (2.3)</td>
<td>6.1 (2.1)</td>
<td>4.4 (1.6)</td>
<td>0.1150</td>
</tr>
<tr>
<td>Range of Follow Up in Years</td>
<td>1.2–9.6</td>
<td>1.2–9.6</td>
<td>1.7–9.4</td>
<td>2.5–6.1</td>
<td>-</td>
</tr>
<tr>
<td>Percent Weight Loss at 1 Year</td>
<td>34.6 (9.3)</td>
<td>32.9 (8.7)</td>
<td>38.2 (9.5)</td>
<td>46.2 (−)</td>
<td>0.0481</td>
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<tr>
<td>Percent Weight Loss at 2 year</td>
<td>36.6 (10.5)</td>
<td>33.7 (8.7)</td>
<td>41.1 (11.7)</td>
<td>52.7 (5.5)</td>
<td>0.0004</td>
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<tr>
<td>Percent Weight Loss at Nadir</td>
<td>38.6 (10.9)</td>
<td>35.8 (9.2)</td>
<td>43.7 (12.4)</td>
<td>51.3 (5.9)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Percent Weight Loss at final Observation</td>
<td>32.3 (12.6)</td>
<td>29.2 (10.7)</td>
<td>38.7 (14.0)</td>
<td>42.9 (13.6)</td>
<td>0.0008</td>
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<tr>
<td>Height (in)</td>
<td>65.7 (3.5)</td>
<td>65.9 (3.5)</td>
<td>65.0 (3.4)</td>
<td>66.8 (3.4)</td>
<td>0.4563</td>
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<td>Age at Surgery</td>
<td>47.8 (10.9)</td>
<td>47.4 (11.1)</td>
<td>50.0 (10.1)</td>
<td>39.8 (8.8)</td>
<td>0.1901</td>
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<tr>
<td>Weight at Surgery (kg)</td>
<td>137.8 (26.3)</td>
<td>134.5 (22.9)</td>
<td>141.6 (29.1)</td>
<td>166.1 (44.3)</td>
<td>0.0430</td>
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<td>Northwestern Hospital, n(%)</td>
<td>74 (77.9)</td>
<td>54 (83.1)</td>
<td>18 (69.2)</td>
<td>2 (50.0)</td>
<td>0.1384</td>
</tr>
<tr>
<td>Male, n(%)</td>
<td>14 (14.7)</td>
<td>10 (15.4)</td>
<td>3 (11.5)</td>
<td>1 (25.0)</td>
<td>0.7525</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are presented as mean(SD) unless otherwise noted.

<sup>b</sup>Tests of association are either ANOVA or non-parametric Kruskal-Wallis test for continuous variables and Pearson’s Chi-Square test for categorical variables.

<sup>c</sup>Only one patient had an observation between 1 and 1.5 years of surgery.

The complete list of SNPs successfully genotyped includes rs10150332, rs10195252, rs1055144, rs10767664, rs10938397, rs10968576, rs12444979, rs1294421, rs13107325, rs1514175, rs1555543, rs206936, rs2112347, rs2241423, rs287019, rs2815752, rs2867125, rs2890652, rs29942, rs3817334, rs4771122, rs4846567, rs4929949, rs543874, rs571312, rs6784615, rs6905288, rs7138803, rs7359397, rs887912, rs9491696, rs9842222, rs987237, and rs9939609.
Table 2

Association of SNP rs4771122 and different measures of weight loss after bariatric surgery

<table>
<thead>
<tr>
<th>Measure</th>
<th>Effect size</th>
<th>P-value for SNP association</th>
</tr>
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<tr>
<td>Percent weight loss 1–9 years after surgery by repeated measures regression(^a)</td>
<td>4.87</td>
<td>.004</td>
</tr>
<tr>
<td>Percent weight loss at 1 year(^b)</td>
<td>3.42</td>
<td>.12</td>
</tr>
<tr>
<td>Percent weight loss at 2 years(^b)</td>
<td>5.37</td>
<td>.004</td>
</tr>
<tr>
<td>Percent weight loss at weight loss nadir(^b)</td>
<td>4.71</td>
<td>.009</td>
</tr>
<tr>
<td>Trajectories of weight loss by proc traj(^c)</td>
<td>Not applicable(^c)</td>
<td>.024</td>
</tr>
</tbody>
</table>

\(^a\) Calculated in a linear mixed regression model, with an unstructured covariance matrix to account for repeated measures, adjusted for gender, surgery age, surgery weight, height, time since surgery, the interaction of surgery age and time since surgery, and recruitment center. Effect size represents increase in long-term percentage weight loss per copy of the minor allele of rs4771122.

\(^b\) Calculated in a linear regression model adjusted for gender, surgery age, surgery weight, height, and recruitment center. Effect size represents increase in percentage weight loss (at 1 or 2 years) per copy of the minor allele of rs4771122.

\(^c\) Comparing the distribution of rs4771122 genotypes across 4 weight loss trajectories determined by proc traj using a chi-squared test. There is a higher percentage of individuals with the GG genotype in the 2 trajectories indicating higher long term weight loss.