Association of *TCF7L2* Allelic Variations with Gastric Function, Satiation, and GLP-1 Levels

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Abstract

Objective: Genetic variation in transcription factor 7-like 2 *(TCF7L2),* a regulator of proglucagon processing, is reproducibly associated with type 2 diabetes. GLP-1 alters gastric function and increases satiation.

Hypothesis: Genetic variation in *TCF7L2* is associated with satiation, gastric motor function, and GLP-1 concentrations.

Methods: In 62 adults, a single nucleotide polymorphism (SNP) of *TCF7L2* (rs7903146) was genotyped and associations with gastric emptying (GE) of solids and liquids, gastric volume (GV), and satiation (maximum tolerated volume and symptoms after nutrient drink test) were explored using a dominant genetic model, with gender and BMI as covariates. In 50 of the participants, we also measured plasma GLP-1 during fasting and after ingestion of a nutrient drink.

Results: Presence of the T allele compared to CC genotype in rs7903146 SNP of the *TCF7L2* gene was associated with reduced fasting GV (246.3 ± 11.4 mL for CC group, compared to 215.7 ± 11.4 mL for CT/TT group, $p = 0.05$) and accelerated GE $t_{1/2}$ of liquids (26.3 ± 2.0 minutes for CC compared to 17.7 ± 1.4 for CT/TT, $p < 0.005$). There was no significant association of rs7903146 SNP with GE of solids, gastric accommodation, satiation, fasting, or postprandial GLP-1.

Conclusion: Our data suggest *TCF7L2* is associated with altered gastric functions that may predispose to obesity. Clin Trans Sci 2011; Volume 4: 183–187

Keywords: gastric emptying, gastric volume, obesity, prediabetes, *TCF7L2*

Introduction

An individual's weight status is at least in part determined by the control of food intake.[1] The stomach signals satiation and other symptoms during feeding in response to ingested volume and calories.[2,3] The postprandial change in gastric volume is an independent predictor of maximum tolerated volume (MTV) and therefore satiation.[4] After administration of pharmacological perturbations to induce postprandial discomfort including fullness and bloating, we demonstrated that gastric emptying of solids at 1 and 4 hours, and fasting gastric volume were associated with postprandial symptom scores reflecting satiation.[5,6]

The incretin hormone, glucagon-like peptide-1 (GLP-1), arises by posttranslational processing of the glucagon gene (*GCG*) product, proglucagon. The transcription factor 7-like 2 gene (*TCF7L2*) may regulate *GCG* expression in the intestinal L cells[7] that secrete GLP-1. GLP-1 is a powerful insulin secretagogue, which also suppresses glucagon secretion. In addition, it delays gastric emptying but does not result in increased satiation possibly because it increases gastric volume and compliance.[8,9] Exogenous GLP-1 or GLP-1 receptor agonists delay gastric emptying and are associated with weight loss.[10] Genetic variation in *TCF7L2* (rs7903146), which is located on chromosome 10q25.2 is associated with type 2 diabetes in different populations.[11,12] Diabetes risk is conferred by the (T) allele of *TCF7L2* (rs7903146).[11–17] The TCF7L2 gene product is a high-mobility group box—containing transcription factor that has a role in the Wnt signaling pathway that is key to developmental and growth regulatory mechanisms of the cell.[11] *TCF7L2* genotype may regulate proglucagon gene and GLP-1 secretion. Variants within the same locus may be associated with BMI and altered concentrations of ghrelin and leptin in males.[13] In depth resequencing in multiple different populations have demonstrated that, although it is an intronic single nucleotide polymorphism (SNP), rs7903146 is either the etiologic variant or it is in such tight linkage disequilibrium that it is indistinguishable from the etiologic variant.[13] The location of this SNP is within a TCF7L2-binding site and may affect TCF7L2-directed regulation of its parent gene.[18] There is also evidence that the SNP rs7903146 variant in *TCF7L2* impacts responsiveness to incretins, such as infused GLP-1.[19,20] Thus, the literature supports the notion that *TCF7L2* (rs7903146) is functionally relevant.

The mechanisms by which this polymorphism may be associated with obesity and its association with altered gastric function are not clearly understood. The aim of this study is to explore the hypothesis that *TCF7L2* polymorphism is associated with changes in gastric function, satiation, and plasma GLP-1 response to a nutrient drink.

Materials and Methods

Participants

The selection of 62 Caucasian participants for this study has been thoroughly described elsewhere.[21] These participants between the ages of 18 and 65 years, residing in Olmsted County, Minnesota had previously enrolled in studies of the pharmacogenetics of sibutramine[22] and of gastric sensorimotor functions in obesity.[4,23] They were a subset of a cohort of 234 normal weight (NW), overweight (OW), or obese (Ob) subjects. We obtained approval to use stored DNA by consent from the patient at the time of original donation, which included permission to conduct future research on the control mechanisms of obesity; the Mayo Clinic Institutional Review Board approved use of the DNA and medical records in accordance with written informed consent from the participants in prior studies to use their stored DNA. One DNA sample was insufficient for assay; therefore this study is based on 61 men and women. Selection criteria for participants were

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as for the original studies: Participants were excluded if they had a positive history of any systemic disease that could affect gastrointestinal motility; or used medications that may alter gastrointestinal motility, appetite, or absorption (e.g., orlistat). Permissible medications were multivitamins, birth control pills, estrogen, and thyroxine replacement, all at stable doses for at least 30 days prior to the studies. Women of childbearing potential (not postmenopausal/not surgically sterilized) had a negative pregnancy test within 48 hours before the studies that included radioisotopes. The study was approved by the Mayo Clinic Institutional Review Board.

Experimental protocol

All participants gave written informed consent. On different days, they presented to the Mayo Clinic Clinical Research Unit at 7 am after an 8-hour fasting period and underwent a gastric emptying scintigraphic study, a nutrient drink satiation test, and a gastric accommodation single photon emission computed tomography (SPECT) study in this order.[21] Gastric emptying and SPECT studies were performed at least 72 hours apart in order to avoid interference by ^{111}In(indium) from the meal ingested during the gastric emptying study with the measurement of gastric volume by SPECT.

Gastric emptying of solids and liquids using scintigraphy

1.0 mCi ^{99m}Tc (technetium)-sulfur colloid was added to two raw eggs during the scrambling, cooking process. The scrambled eggs were served on one slice of buttered bread with 240 mL of 1% milk labeled with 0.1 mCi ^{111}In-DTPA. The total caloric value of the meal was 296 kcal, and the composition of macronutrients was 32% protein, 35% fat, 33% carbohydrate. Anterior and posterior gamma camera images were obtained immediately after radiolabeled meal ingestion, every 15 minutes for the first 2 hours, then every 30 minutes for the next 2 hours [total 4 hours after the radiolabeled meal].[4] Data were analyzed as in previous studies.[24] Geometric mean of counts in gastric regions of interest drawn on the anterior and posterior images were used (after correction for radioisotope decay) to estimate the proportion of ^{99m}Tc or ^{111}In emptied from the stomach at each time point. Gastric emptying endpoints of analysis were $t_{1/2}$ for solids and liquids, which were estimated by linear interpolation of the data at each time point.

Gastric volume and accommodation assessment with ^{99m}Tc-SPECT

We measured gastric volume during fasting and during the first 30 minutes after 300 mL of Ensure (calorie content 316 kcal) (Abbott Laboratories, Abbott Park, IL, USA) using noninvasive single photon emission computed tomography. The method has been previously validated.[25] Intravenous injection of ^{99m}Tc sodium pertechnetate, which is taken up by the parietal and nonparietal cells of the gastric mucosa, allows visualization of the stomach wall. Tomographic images of the gastric wall are obtained throughout the long axis of the stomach using a dual-head gamma camera (SMV SPECT System*, Twinsburg, OH, USA) that rotates around the body. The radiolabeled circumference of the gastric wall (rather than the intragastric content) is thus identified. Using the AVW 3.0 (Biomedical Imaging, Mayo Foundation, Rochester, MN, USA) image processing libraries, a three-dimensional rendering of the stomach is obtained and its volume (mL) calculated.

There is high intraobserver reproducibility to measure gastric volume with this technique.[26] The interindividual coefficient of variation (COV_{INTER}) for all subjects in a study from our laboratory (n = 433) was 32.6% fasting, 16.0% fed, and 19.0% fed – fasting. The intraindividual COV (COV_{INTRA}) for 47 subjects with repeat estimates of gastric volume was 37.0% fasting, 17.6% fed, and 22.0% fed – fasting. COV_{INTRA} was stable over a time interval from 2 to 60 months. There are no significant differences by gender.[27]

Nutrient drink satiation test

We used a validated nutrient drink test[28] to measure satiation and postprandial symptoms when drinking a liquid nutrient at constant rate of 120 mL every 4 minutes (Ensure 1 kcal/mL, 11% fat, 73% carbohydrate, and 16% protein). Participants scored the time needed to reach each level of fullness using a digital timer. Every 5 minutes, participants scored level of fullness or satiation using a scale combining verbal descriptors and numbers (0 = no symptoms; 5 = maximum or unbearable fullness/satiation). Nutrient intake was stopped when subjects reached the score of 5, with maximum satiation indicated by the MTV of Ensure. Postprandial symptoms of fullness, nausea, bloating, and pain, were measured 30 minutes after the meal using 100-mm horizontal visual analog scales, with the words "none" and "worst ever" anchored at the left and right ends of the lines for each symptom.

Plasma GLP-1 measurement

GLP-1 was measured as the biologically active GLP-1 (7–36, 7–37) using a two-site noncompetitive immunoassay based on enzyme-labeled quantification of GLP-1 detected by a fluorogenic substrate (Millipore Inc., Billerica, MA, USA).

Plasma GLP-1 was measured at 5 minutes before nutrient drink meal ingestion (baseline), 0 (beginning of feeding), 15, 30, 45, 60, and 120 minutes after starting the nutrient drink satiation test.

Determination of genotypes

DNA was extracted from whole blood as previously described. Genotyping of *TCF7L2* (*rs7903146*) was performed using Taqman SNP Genotyping assays (Applied Biosystems Inc., Foster City, CA, USA) in accordance with the manufacturer's instructions.

Statistical analysis

All data are presented as means ± SEM, or percentages, as noted. The univariate associations of subject characteristics and response measures (e.g., gastric emptying $T_{1/2}$ values) with overall genotype were assessed using Fisher's Exact test (e.g., association with categorical variables like gender) and for quantitative traits, the Kruskal-Wallis test assuming a general allelic model for the genotype and pair-wise associations using the Wilcoxon rank sum test based on the dominant genetic model (i.e., pooling the minor allele homozygote with the heterozygote genotype: TT plus CT versus CC for the *TCF7L2*). This grouping is based on prior demonstration that the disease-predisposing allele is the T allele for *TCFL2* genotype. This assumes the single allele has a biological effect. The association of genotype with physiologic response measures were also assessed using an analysis of covariance adjusting for gender and BMI.

A sample size assessment for detecting clinically relevant associations for the *TCF7L2* genotype was done by estimating the differences between two groups (i.e., assuming a dominant genetic model with CC vs. CT/TT) that could be detected given the observed variation in the measured responses and the number of subjects that were obtained in each genotype group (27 CC vs. 34 CT/TT for *TCF7L2*). The differences between groups that could be detected with approximately 80% power (two-sided

	SD	Mean (group 1)	Mean (group 2)
Fasting gastric volume, mL	64	230	277
Δ Postprandial accommodation volume, mL	85	490	553
Aggregate symptom score (maximum 400)	72	150	203
Maximum tolerated volume, mL	312	1,210	1,440
Gastric emptying of solids $T_{1/2}$, min	36	120	147
Gastric emptying of liquids $T_{1/2}$, min	9.7	22	30

The table shows differences between two *TCF7L2* genotype groups that could have been detected with approximately 80% power (two-sided $\alpha = 0.05$) using a two-sample *t*-test (assuming the observed pooled standard deviation [SD] listed in the table).

Table 1. Sample size assessment.

	TCF7L2		
Genotype	CC	CT	TT
N	27	31	3
Females,%	77.8	64.5	66.7
Age, Years	45 ± 2	43 ± 2	45 ± 2
Weight, Kg	88.4 ± 3.0	86.0 ± 2.8	90.4 ± 7.5
BMI, Kg/m^2	31.3 ± 0.8	30.4 ± 0.9	32.0 ± 2.9

†Fisher's exact test applied for comparisons of gender proportion; Kruskal-Wallis test used for comparison of age, weight, and BMI.

Table 2. Subject characteristics by genotype (summary values are mean ± SEM, unless otherwise indicated).†

	TCF7L2	
Genotype	CC	CT/TT
N	27	34
Gastric emptying solids, t1/2, min	126.0 ± 8.0	115.9 ± 5.5
Gastric emptying liquids, t1/2, min	26.3 ± 2.0**	17.7 ± 1.4
Fasting volume, mL	246.3 ± 11.4*	215.7 ± 11.4
Δ Postprandial accommodation volume, mL	482.1 ± 16.7	491.6 ± 15.6
Maximum tolerated volume, mL	1221.5 ± 56.7	1211.4 ± 56.6
Aggregate symptom score, mm	155.4 ±13.0	145.7 ± 13.2
Fasting GLP-1, pmol/L	3.5 ± 0.6	4.5 ± 1.1
Postprandial GLP-1, pmol/L	6.9 ± 0.7	8.7 ± 1.0

**$p < 0.005$; *$p = 0.05$.

Table 3. Gastric motor, sensory functions and GLP-1 values by the dominant genetic model.

α level of 0.05) using a two-sample *t*-test (assuming the listed pooled standard deviation [SD]) are listed in *Table 1*. Except for gastric emptying (two endpoints), each physiologic response corresponds to a distinct null hypothesis (e.g., association of genotype of interest with nutrient drink test MTV, i.e., satiation). The control mechanisms for the different physiological functions such as gastric emptying and accommodation are different. For example, gastric emptying is critically dependent on cholinergic mechanisms, and gastric accommodation on nitrergic control; vagal afferent function is essential for sensation, while efferent function controls gastric motor functions. In addition, the total variance in postprandial satiation that can be explained by gastric emptying at 1 and 4 hours and fasting gastric volume is <25%.[5] Therefore, while these different functions are involved in the integrated response to ingestion of a meal, they really represent distinct physiologic functions, each of which is assessed with a distinct null hypothesis when we evaluate associations with multiple intermediate phenotypes. A *p*-value of less than 0.05 was considered to be statistically significant except in the assessment of the association between genotype and gastric emptying (α level of 0.025 in view of two comparisons for solids and liquids).

The association between gastric emptying of solids and liquids was estimated using the Pearson (linear) correlation coefficient and a similar approach was used to assess the correlation between gastric motor functions and postprandial GLP-1 levels. Analyses used the SAS statistical package.

Results

Subject characteristics

Demographic characteristics of individuals in each genotype group are outlined in *Table 2*. There were no significant overall associations of the genotype with age, gender, BMI or weight.

Relationship of *TCF7L2* genotype with gastric motor functions and satiation

Table 3 summarizes the results for each genotype. *TCF7L2* genotype had a modest association with fasting gastric volume ($p = 0.06$ using the general allelic model, and $p = 0.05$ using the dominant genetic model, ANCOVA adjusting for gender), with smaller volumes associated with the T allele compared to people with the CC genotype. There was no significant association with delta volume from fasting that represents gastric accommodation.

As expected, there was a significant correlation between solid and liquid gastric emptying $T_{1/2}$ times ($r = 0.58$, $p < 0.001$). Genotype was also significantly associated with gastric emptying of liquids ($p = 0.007$ by the general allelic model, and $p = 0.002$ by the dominant genetic model, ANCOVA adjusting for gender), with faster emptying in those with a T allele (*Table 2*). It is worth noting that the *p*-value for the association of liquid gastric emptying with *TCF7L2* ($p < 0.01$) was less than an adjusted α level of 0.025, considering the two tests exploring effects on gastric emptying (solid and liquid).

There was no significant association *of TCF7L2* genotype with gastric emptying of solids, or indices of satiation (MTV and postprandial gastric symptoms).

Plasma GLP-1 levels and *TCF7L2* genotypes

Fasting and postprandial GLP-1 data are summarized in *Table 3*. There was no significant association between *TCF7L2*, genotypes with fasting, and postprandial GLP-1 levels.

In general, liquid gastric emptying predicted mean postprandial GLP-1 in response to a nutrient drink test ($p = 0.02$).

Discussion

TCF7L2 gene has been reproducibly associated with defects in insulin secretion, type 2 diabetes, and with fasting and

postchallenge glucose concentrations. We have tested the effect of genetic variation of this gene through the differential effects of the SNP 7903146; as noted in the Introduction, there is evidence that this SNP is functionally relevant. In this study, we explored the association of this gene with gastric motor function and satiation in humans. We demonstrated that the presence of the T allele in the *TCF7L2* genotype (at the SNP site rs7903146) was associated with both fasting gastric volume and gastric emptying of liquids but not with altered fasting or postprandial GLP-1 levels.

Evidence suggests that gastric sensorimotor functions play a role in the development of obesity.[4–6] Satiation, mediated peripherally by the stomach, determines the amount of food intake and there is growing evidence to suggest that there may be a genetic susceptibility to postprandial satiation.[21] The observed accelerated emptying of liquids with *TCF7L2* genotype might predispose to postprandial hyperglycemia as liquids containing calories in solution are more rapidly emptied from a stomach with reduced fasting volume. Low-calorie-density liquids empty exponentially from the stomach and this emptying rate is increased with smaller gastric volumes, as occurs after fundoplication[29–32] or when the stomach is partly filled with a balloon at low constant pressure.[33] Accelerated gastric emptying of liquids is associated with postprandial hyperglycemia;[34] on the other hand, incretins such as GLP-1 or GLP-1 receptor agonists such as exanetide reduce postprandial glycemia in part by increasing insulin secretion, and in part by inhibiting gastric emptying. The lack of association between *TCF7L2* genotype and postprandial gastric volume and emptying of solids suggest that *TCF7L2* does not reduce satiation by affecting gastric emptying and accommodation in response to meal ingestion. *TCF7L2* expression in the mouse brain[35] suggests that central mechanisms controlled by this gene may affect satiation, as well as the known association with diabetes.

The lower fasting gastric volumes and accelerated liquid gastric emptying associated with the T allele of the *TCF7L2* genotype suggest that GLP-1 secretion is driven by nutrient delivery to the mid and lower small intestine. This is supported by the finding that liquid gastric emptying predicted mean postprandial GLP-1 in response to a nutrient drink test. Indeed, isolated L cells secrete GLP-1 in response to the presence of intraluminal nutrient.[36]

On the other hand, we did not find an association between *TCF7L2* genotype and GLP-1 levels. This finding confirms two recent reports: First, Villareal et al. have suggested that the increased risk of diabetes conferred by *TCF7L2* genotype was partly due to a modifying effect of incretins on insulin secretion rather than decreased GLP-1 secretion;[37] second, Schafer et al. showed that *TCF7L2* genotype did not influence postprandial GLP-1 levels after an oral glucose tolerance test.[20]

The strengths of our study include the use of quantitative traits with well-validated methods with known coefficient of variation and robust statistical power to detect influence of variation in the *TCF7L2* gene on gastric functions, satiation, and GLP-1 levels. A limitation of this study is that we did not measure fasting or postprandial blood glucose in response to the standardized meal. This needs to be the focus of future studies especially since it has been demonstrated that the *TCF7L2* genotype predisposes to type 2 diabetes.[17]

In summary, our data suggest that genetic variation in *TCF7L2* is associated with variations in gastric functions that may predispose to obesity. Further studies exploring the role of genetic susceptibility in alterations in gastric functions and glycemic control that may predispose to obesity are warranted. In addition, such genotype-intermediate phenotype association studies may help identify targets for future antiobesity clinical trials.

Conflict of Interest

The authors have no conflicts of interest to disclose.

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