Association between allelic variants in the glucagon-like peptide 1 and cholecystokinin receptor genes with gastric emptying and glucose tolerance

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Abstract

Background.—Nutrient-mediated release of cholecystokinin and glucagon-like peptide-1 (GLP-1) regulate gastric emptying (GE) via duodenogastric feedback mechanisms; GLP-1 also regulates postprandial insulin secretion. Some patients with functional upper gastrointestinal symptoms have impaired glucose tolerance during *enteral* dextrose infusion. Our hypothesis was that variants in *CCK, GLP-1,* and *TCF7L2* (transcription factor 7-like 2 locus), which is associated with greatest genetic risk for development of type 2 diabetes mellitus, are associated with GE and independently with glucose tolerance. Our aims were to evaluate the associations between these GE, glucose tolerance and these SNPs.

Methods.—Genetic variants, scintigraphic GE of solids, plasma glucose, insulin, and GLP-1 during enteral dextrose infusion (75gm over 2 hours), were measured. GE and enteral dextrose infusion were respectively evaluated in 44 (27 controls and 17 patients with functional dyspepsia or nausea) and 42 (28 controls, 14 patients) participants; of these, 51 participants consented to assessment of single nucleotide polymorphisms (SNPs). Four functional SNPs were studied: rs6923761 and rs1042044 at *GLP-1* receptor, rs7903146 *(TCF7L2)*, and rs1800857 *(CCK receptor)*.

Results.—Gastric emptying was normal in 38, rapid in 4, and delayed in 2 participants; 38 had normal and 4 had impaired glucose tolerance. The T allele at rs7903146 *(TCF7L2)* was non-significantly associated (p=0.14) with faster GE. The associations between SNPs and demographic variables, GE t_{half}, glucose tolerance and plasma GLP1 levels were not significant.

Conclusions.—There is a trend toward an association between faster GE and the diabetes-associated allele at rs7903146 in *TCF7L2*. However, these SNPs were not associated with plasma glucose or GLP1 concentrations during enteral dextrose infusion.

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BACKGROUND

Some patients with functional upper gastrointestinal symptoms (i.e, non ulcer dyspepsia [NUD], nausea and/or vomiting, and postprandial abdominal bloating and/or distention) have rapid gastric emptying (GE).(1–5) Among patients who do not have a disease (e.g., diabetes mellitus) or gastric surgery that is associated with rapid GE, the rapid GE is deemed idiopathic.(6–8) Conceivably, disturbances of one or more mechanisms that normally regulate GE, such as impaired gastric accommodation, exaggerated gastric contractility, or impaired duodeno-gastric feedback mechanisms, may cause rapid GE in these patients.(2, 9, 10)

In the small intestine, nutrients evoke neurohumorally-mediated duodenogastric feedback reflexes that inhibit gastric emptying by modulating gastric and pyloric motor activity.(7) Cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), which are released in response to nutrients in the small intestine, delay GE by increasing gastric accommodation and/or inhibiting antral contractility and/or increasing pyloric tone. Rapid GE or dumping induces an exaggerated release of these and other hormones (e.g., insulin and glucagon) that at least partly cause the gastrointestinal and vasomotor symptoms of dumping syndrome. (8) However, in one study, we observed that one-third of non-diabetic patients with NUD had impaired glucose tolerance (IGT) during *duodenal* infusion of glucose (75 gm) at a controlled rate over 2 hours.(9) These patients had IGT despite increased plasma GLP-1 levels during duodenal glucose infusion. Since the nutrients were delivered to the small intestine, the IGT is not secondary to rapid GE. Rather, because GLP-1 partly mediates the postprandial release of insulin and also modulates gastric emptying, we postulated that an impaired response to GLP-1, possibly related to genetic variation at the GLP-1 receptors, may explain IGT and rapid GE in these patients.

Hence, the overall objectives of this sub-study were to evaluate the associations between glucose tolerance and insulin release and selected polymorphisms in genes that control CCK, GLP-1, and other genes that predispose to T2DM and GE among patients with upper gastrointestinal symptoms. The specific aims were to (i) evaluate the associations between single nucleotide polymorphisms (SNPs) in the genes of the CCK and GLP1 receptors and GE, (ii) better understand the mechanisms of hyperglycemia by comparing the insulin secretion rate in patients with normal and impaired glucose tolerance, and (iii) assess the associations between insulin secretion rate and the genes of GLP-1 receptor and TCF7L2 which are associated with regulation of glycemia.

MATERIALS AND METHODS

Study Design

The original study was designed to evaluate hormonal responses to enteral dextrose infusion in 35 healthy asymptomatic controls and 30 patients with functional upper gastrointestinal (GI) symptoms (dyspepsia or nausea and vomiting) by Rome III criteria; gastric emptying of solids was also evaluated in 25 healthy controls and 30 patients.(9) From that cohort, 34 controls (23 women, age, 41 [3] years, mean [SE]; BMI 26.4 (0.7) kg/m^2) and 17 patients (11 women, 45 [5] years, BMI 26.2 (1.2) kg/m^2) consented to genotyping and are included in this paper. Among all participants, exclusion criteria were age <18 or >70 years; diabetes mellitus; a structural disorder affecting the GI tract; clinically significant systemic (eg, cardiorespiratory) disease that may interfere with study objectives or pose safety concerns, or both; medications that can affect GI motility; GI surgery other than appendectomy, cholecystectomy, hysterectomy, tubal ligation, or inguinal hernia repair; or a hemoglobin level <12.9 g/dL in men and <11.5 g/dL in women. All women of child-bearing potential had to have a negative pregnancy test within 48 hours of study participation. The Mayo Clinic Institutional Review Board approved the study. All participants signed informed consent.

Assessment of Dyspepsia Symptoms—Dyspepsia symptoms and quality of life (QOL) were evaluated with the Nepean Dyspepsia Index questionnaire (11, 12). The Hospital Anxiety and Depression Scale (HADS) questionnaire documented anxiety and depression (13).

Gastric and Small-Bowel Transit—On average, the assessments (i.e., gastric emptying and enteral nutrient infusion) were separated by 8 days. Gastric emptying of a solid meal (296 kcal; 32% protein, 35% fat, and 33% carbohydrate) consisting of 2 eggs labeled with technetium Tc 99m sulfur colloid (1 mCi) served on 1 slice of bread with milk (240 mL; 1%) was evaluated with scintigraphy.(14) Rapid and delayed gastric emptying were defined relative to 10th through 90th percentile values in controls from the present study. *Rapid gastric emptying* was defined as emptying >90th percentile value at 60 or 120 minutes (>31.4% and >71.2%, respectively for women and >40% and >82% for men) or half-time (t$_{50}$) less the 10th percentile value (i.e., <89 and <73.2 minutes for women and men, respectively). *Delayed gastric emptying* was defined as emptying <10th percentile value at 2 (i.e., <25% for women and <28% for men) or 4 hours (i.e., <76% for women and <77% for men) or t$_{50}$ longer than the 90th percentile value (i.e., >180 and >165 minutes for women and men, respectively).

Enteral Nutrient Infusions—Through a 8-Fr nasoduodenal feeding tube (Abbott Nutrition) the tip of which was placed with fluoroscopic guidance in the second part of the duodenum, dextrose (Limeondex, 75gm diluted to 222 ml, 300 kcal, Therma Fisher Scientific Inc) was administered over 2 hours at the rate at which glucose is absorbed after oral ingestion. (15) (16–18)

Glycemia and Enteral Hormonal Measurements—During enteral nutrient infusion, blood samples for measurement of plasma glucose, C-peptide, glucagon, GLP1, CCK, and PYY were collected at 5-minute intervals for 30 minutes, at 10-minute intervals from 30 to 60 minutes, and at 15-minute intervals from 60 to 120 minutes. Arterialized venous plasma samples were obtained from a retrograde hand or forearm vein and were placed in a Perspex hot box heated to 55 °C. These samples were placed in ice, centrifuged at 4°C, separated, and stored at −20°C until assayed. Glucose was measured by the Hitachi 912 assay (Roche Diagnostics). An enzyme-linked immunosorbent assay (ELISA) (Linco Research, Inc) was used to measure biologically active GLP1(7–36 amide, 7–37) concentrations. The CCK immunoassay (Alpco Diagnostics) assesses most biological active forms with nearly equimolar potency.(19) Glucagon was measured with a direct, double-antibody. radioimmunoassay (Linco Research, Inc). C-peptide levels were measured with a 2-site immunometric (sandwich) assay using electrochemiluminescence detection (Cobas e411; Roche Diagnostics).

Genotyping—Genomic DNA was isolated from whole blood by use of the QIAamp DNA Blood Maxi Kit (Qiagen, Valencia, CA), and stored at −80°C until genotyping. Candidate genotypes were analyzed with established PCR-based methods i.e., rs7903146 (TCF7L2), (20) rs1800857,(21) and with Taqman assays (catalog # C___2491143_10) (catalog C__25615272_20) (Applied Biosystems, Grand Island, NY) for GLP1R SNPs rs1042044 and rs6923761. Following polymerase chain reaction amplification, end reactions were analyzed by using ABI 7300 Real-Time PCR System by Sequence Detection Software (Applied Biosystems, Grand Island, NY). The PCR products were treated with ExoSAP-IT For PCR Product Cleanup (Affymetrix, Santa Clara, CA) and sequenced at the Mayo Clinic Genome Facility on an ABI PRISM™ 3730xl DNA Analyzers (Applied Biosystems, Grand Island, NY).

The SNPs evaluated in this study have been linked with the control of blood glucose (i.e., *GLP1* and *TCFL2*) and/or hormones (i.e., *CCK*) that regulate gastric emptying. At the *GLP1R* gene, 2 coding variants (i.e., rs6923761 and rs1042044) have been associated with altered GLP1R function. Subjects who are homozygous for the major allele of rs6923761 secreted significantly more insulin than subjects containing at least one copy of the minor allele.(22) At rs1042044, the homozygous minor allele is linked to increased morning cortisol levels in children.(23)

The diabetes-associated T allele of the intronic SNP rs7903146 located within the transcription factor 7 like 2 (*TCF7L2*) gene affects insulin secretion and β-cell function,(24) and is the strongest genetic association with type 2 diabetes mellitus,(25) increasing the risk of type 2 diabetes by about 50%.(26) Also germane to this study, the T allele was previously shown to be associated with reduced fasting gastric volume and faster GE of liquids.(20) Finally, for the *CCK receptor* gene, we evaluated the intronic SNP rs1800857, where the wild T allele is associated with slow GE (27) and with constipation-predominant and mixed irritable bowel syndrome compared with healthy controls.(28)

Insulin Secretion Rate—Insulin secretion rate was calculated by deconvolution from C-peptide concentrations using a two-compartment model of C-peptide kinetics, where the

relevant kinetic indices were estimated using anthropometric data as previously described (29) and recently validated independently.(30)

Data and Statistical Analysis

The associations between SNPs and physiological variables employed a dominant model in which homozygotes for the major allele were compared with homozygotes for the minor allele grouped with the heterozygotes. The associations between these SNPs and gastric emptying summarized as normal versus rapid and separately normal versus delayed was evaluated with Fischer's exact test. The Wilcoxon rank sum test evaluated the relationship between the t_{50} and SNPs. Linear regression models evaluated the association between each SNP (predictor variables) to the area under the curve (AUC) for glucose and separately ISR (dependent variables). All analyses were performed with SAS (SAS Institute, Cary, NC) The data are summarized as Mean ± SD.

RESULTS

Baseline Characteristics and Study Flow

Among 17 patients, 15 and 2 satisfied Rome III criteria for functional dyspepsia and nausea respectively. Of 51 participants (34 controls and 17 patients) in whom genotyping was performed, gastric emptying was evaluated in 44 (27 controls and 17 patients) and the enteral dextrose infusion was performed in 42 participants (28 controls and 14 patients) (Figure 1). Gastric emptying and enteral dextrose infusion were both evaluated in 36 participants (22 controls and 14 patients).

On a scale from 0 (worst symptom severity) to 13 (no symptoms), the Nepean Dyspepsia Index mean symptom severity score was 12.9 ± 0.2 in controls and 11.3 ± 0.8 in patients ($P<.0001$). The QOL scores were 99.8 ± 1.0 in controls and 50.3 ± 18.1 in patients ($P<.0001$), on a scale from 0–100 where lower scores represent poorer QOL.

Distribution of SNPs and Associations with Demographic Features

For rs 1800857 (*CCK*), 40 participants were homozygotes for the major allele and 11 were heterozygotes. For rs 7903146 (*TCF7L2*), 28 and 3 participants were homozygous respectively for the major and minor alleles; the remainder (n=20) were heterozygotes. For rs 1042044 (*GLP-1 receptor*), there were 27 heterozyogotes and 24 homozygotes - 16 for the major and 9 for the minor allele. For rs 6923761 (*GLP-1*), there were 33 homozygotes - 27 for the major and 6 for the minor allele – and 18 heterozygotes. Associations between the SNPs and demographic variables (i.e. age, sex, and BMI) and with participant status (ie, control or patient) were not significant. Table 1 shows the distribution of demographic features and gastric emptying values for major and minor alleles only among participants in whom gastric emptying was evaluated.

Gastric emptying and associations with SNPs

Gastric emptying of solids was normal in 38 participants (ie, 27 controls and 11 patients). The remaining 6 patients had rapid (4 patients) or delayed GE (2 patients). Gastric emptying

of liquids was normal in 27 controls and 14 patients; 2 had rapid and 1 had delayed GE (data not shown).

For *TCF7L2*, the minor allele was associated with numerically shorter GE t_{half} (i.e., faster GE) for solids (p=0.14) and liquids (p=0.15) compared to the major allele (Tables 1 and 2). However, differences were not significant. The other 3 SNPs were not associated with faster (Wilcoxon rank sum test) or with rapid GE (Fisher's exact test).

Enteral dextrose infusion and associations with SNPs

During the enteral dextrose infusion, the blood glucose concentration (area under the curve) exceeded the 90th percentile values in controls in 4 of 14 (29%) patients, indicative of impaired glucose tolerance. The insulin secretion rate could be reliably estimated in 32 participants (ie, 23 controls and 9 patients). The plasma glucose, GLP-1 levels, and insulin secretion rate were not significantly associated with any of the selected SNPs (Table 3, Figure 2).

DISCUSSION

Our study shows that the T allele at rs7903146 (*TCF7L2*) is associated with numerically faster GE of liquids and solids in patients with NUD. This is consistent with prior observations among 62 normal weight, overweight, or obese individuals previously evaluated at our institution, which showed the T allele at the *TCF7L2* rs7903146 polymorphism was significantly associated with faster GE of liquids but not solids.(20) *TCF7L2*, a member of the Wnt signaling pathway, is involved in β-cell development during embryogenesis and also protects mature β-cells.(31) The T allele at the *TCF7L2* rs7903146 polymorphism is associated with impaired glucose-stimulated and incretin-induced insulin secretion (32–34) and conversion of proinsulin to insulin.(35) Glucose-stimulated insulin secretion is partly mediated by GLP-1. The T allele at the *TCF7L2* rs7903146 polymorphism is nominally associated (p=0.06) with smaller fasting gastric volumes.(20) Taken together, these data are consistent with the hypothesis that among people with the T allele, lower gastric volumes predispose to higher gastric pressure and faster GE. This hypothesis can be tested in future studies by studying whether the *TCF7L2* rs7903146 polymorphism predicts the effects of GLP-1 on fasting and postprandial gastric volumes in healthy people, NUD, and diabetes mellitus. A separate but related question is whether this polymorphism is associated with rapid GE in patients with type 2 DM.(6)

A second mechanism of interest because of its effects on gastric functions and glycemic control is GLP-1 which increases fasting and postprandial gastric volumes; the latter is partly mediated by nitric oxide.(36) However, GE of solids, gastric accommodation, and fasting and postprandial plasma GLP-1 levels measured over 120 minutes during enteral dextrose infusion were not significantly associated with *GLP-1* polymorphisms.

None of the other SNPs were associated with GE. While the associations between the two SNPs in *GLP-1* and GE have not been evaluated previously, the major (T) allele at rs1800857 *(CCK)* was associated, albeit weakly, with delayed GE among patients with constipation-predominant irritable bowel syndrome.(27) The SNPs rs6923761 and

rs1042044 in the *GLP-1* gene which are associated respectively with GLP1-induced insulin secretion (22) and cortisol levels were not associated with abnormal GE in this study.

Peripheral insulin concentrations represent the end result of insulin secretion and hepatic extraction of insulin. Hence we used a validated approach (i.e., deconvolution of C-peptide concentrations) to measure insulin secretion rates. When caloric appearance into the duodenum is controlled by enteral infusion (i.e., not affected by differences in GE among patients), insulin secretion rate did not differ between groups. However it is possible that accelerated gastric emptying, which is associated with lower plasma insulin concentrations in type 2 diabetes mellitus, might unmask an inherent defect in the beta-cell response.(6) Also, we only measured insulin secretion. The gold-standard measurement of beta-cell function in a given individual expresses insulin secretion as a function of the prevailing insulin action.(37) It is therefore possible that our methodology missed significant between-group differences in beta-cell function.

The lack of associations between these selected SNPs and biological variables (gastric emptying, glucose and GLP1 during enteral dextrose infusion) tested in this study may reflect a type 2 error. A *post hoc* analysis of our dataset suggests that for the *TCF7L2* rs7903146 polymorphism, assuming a SD of 49 minutes and a sample size of 40 participants we had 80% power (two-sample t test, two-sided $\alpha = 0.05$) to detect a difference of 44 minutes in the GE of solids between the major and minor alleles. A sample size of 263 participants would be necessary to determine if the observed difference of 17 minutes between the major and minor alleles is statistically significant. One hundred and twenty three participants would be required to detect the minimum clinically-important difference defined as 0.5 times the baseline SD (i.e., 49 minutes) i.e., 25 minutes.(38)

There were several strengths and limitations of this prospective study. Gastric emptying, and measurements during enteral dextrose infusion were obtained with state-of-the-art techniques and compared in healthy controls and patients with NUD. For all SNPs, the observed minor allele frequency was comparable to previous studies, i.e., observed (published) were 0.11 (versus 0.13) for rs1800857, 0.27 (0.28) for rs7903146, 0.43 (0.40) for rs1042044, and 0.30 (0.37) for rs6923761. However, the pilot study was under-powered to detect clinically-significant differences between groups.

In conclusion, the current results provide the preliminary data for future studies evaluating the relationships between genetic polymorphisms, gastric emptying, and glycemia during enteral glucose infusion.

ACKNOWLEDGEMENTS

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KEY POINTS

- For reasons unclear, some patients with functional upper gastrointestinal symptoms have impaired glucose tolerance during *enteral* glucose infusion.

- This study evaluated the associations between selected genetic polymorphisms in *CCK, GLP-1,* and *TCF7L2* and gastric emptying (GE), glucose tolerance, and insulin release in such patients.

- While studied polymorphisms were not associated with plasma glucose, GLP-1 levels, and insulin secretion rates, a trend toward an association between faster GE and a diabetes-associated allele in *TCF7L2* was observed.

- This study serves as a pilot for future studies evaluating the associations between genetic polymorphisms, GE, and glycemia.
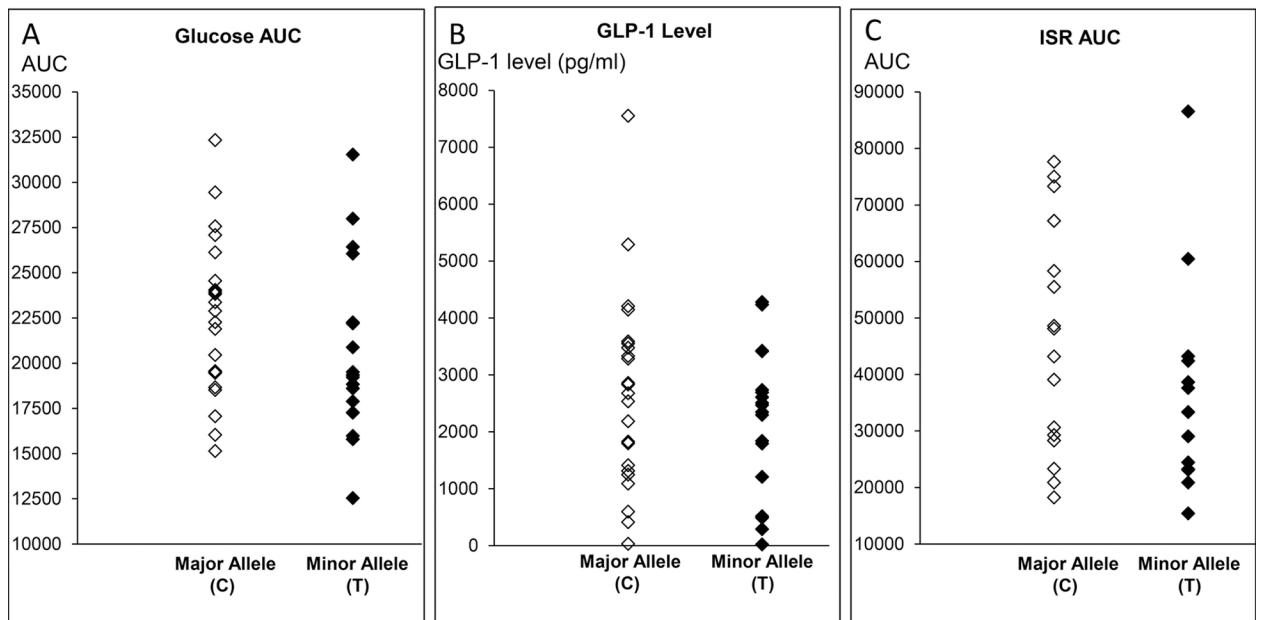
Figure 1. Experimental Design.

The physiological assessments were performed in 35 controls and 30 patients. The insulin secretion rate could be accurately estimated in 23 controls and 9 patients.
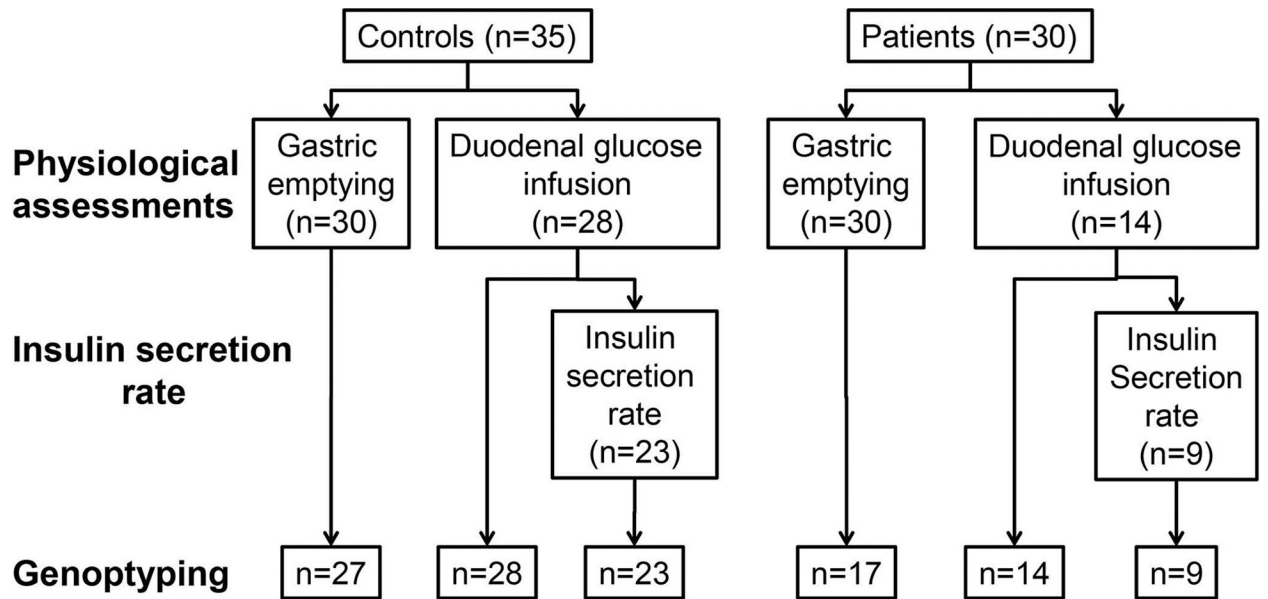
Figure 2. Comparison of plasma glucose (panel A), GLP-1 levels (panel B), and insulin secretion rate (panel C) versus major and minor alleles at rs 7903146 *(TCF7L2)*.

Table 1.

Associations between SNPs, Demographic Features, and Gastric Emptying

SNP (gene)	Allele	N	Age	Females (%)	BMI Kg/m²	GE t_half solids (min)	GE t_half liquids (min)	GE at 60 minutes (%)	GE at 120 minutes (%)	GE at 240 minutes (%)
rs1800857 (CCK)	Major	34	42 ± 17	68	26 ± 4	113 ± 35	28 ± 26	27 ± 15	60 ± 19	95 ± 9
	Minor	10	45 ± 16	64	29 ± 5	122 ± 39	24 ± 11	20 ± 10	53 ± 19	92 ± 14
Rs 7903146 (TCF7L2)	Major	24	45 ± 17	68	27 ± 5	122 ± 37	32 ± 28	21 ± 9	53 ± 19	92 ± 12
	Minor	20	39 ± 16	65	26 ± 4	105 ± 31	21 ± 14	30 ± 16	64 ± 19	97 ± 6
rs1042044 (GLP1 receptor)	Major	12	41 ± 18	73	27 ± 4	116 ± 39	19 ± 10	22 ± 10	58 ± 19	95 ± 9
	Minor	32	43 ± 16	64	26 ± 5	114 ± 35	31 ± 26	26 ± 15	58 ± 19	96 ± 8
rs6923761 (GLP1)	Major	23	41 ± 16	63	26 ± 5	117 ± 38	29 ± 28	27 ± 16	58 ± 20	95 ± 5
	Minor	21	44 ± 18	71	26 ± 4	113 ± 33	26 ± 18	23 ± 9	59 ± 18	94 ± 11

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Table 2.

Distribution of SNPs in Participants with Normal, Rapid and Delayed Gastric Emptying[1]

SNP (gene)	Allele	N	GE solids		
			Normal	Rapid	Delayed
rs1800857 (CCK)	Major	34	30 (88%)	3 (9%)	1 (3%)
	Minor	10	8 (80%)	1 (10%)	1(10%)
Rs 7903146 (TCF7L2)	Major	24	21 (88%)	1 (4%)	2 (8%)
	Minor	20	17 (85%)	3 (15%)	0
rs1042044 (GLP1 receptor)	Major	12	10 (84%)	1(8%)	1(8%)
	Minor	32	28 (88%)	3 (9%)	1 (3%)
rs6923761 (GLP1)	Major	23	19 (83%)	3 (13%)	1 (4%)
	Minor	21	19 (90%)	1 (5%)	1 (5%)

Because the normal values for GE were derived from healthy controls, all healthy controls had normal GE.

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Table 3.

Distribution of SNPs versus Glycemia, GLP1 Levels, and Insulin Secretion Rates during Enteral Dextrose Infusion

SNP (gene)	Allele		Glucose	GLP1	Insulin Secretion rate
rs1800857 (CCK)	Major	N	34	33	25
		Values	19553 (18509, 24038)	2690 (1826, 3476)	37602 (24432, 49146)
	Minor	N	9	9	7
		Values	20450 (19353, 23935)	1410 (1208, 1838)	43211 (36204, 63796)
Rs 7903146 (TCF7L2)	Major	N	23	23	18
		Values	22265 (19484, 24295)	2675 (1361, 3511)	45627 (29598, 64942)
	Minor	N	20	19	14
		Values	19338 (17572, 22221)	2468 (1499, 3072)	33329 (23535, 43008)
rs1042044 (GLP1 receptor)	Major	N	13	12	10
		Values	21383 (19543, 24089)	2004 (1234, 3367)	35978 (29598, 45835)
	Minor	N	30	30	22
		Values	19479 (18037, 23869)	2641 (1791, 3415)	42784 (23580, 57585)
rs6923761 (GLP1)	Major	N	22	21	17
		Values	19504 (18059, 23749)	2704 (1952, 3421)	43167 (29021, 60434)
	Minor	N	21	21	15
		Values	20663 (19180, 24089)	2004 (1234, 2975)	33329 (25773, 52037)

Values are Median (Q1, Q3)