Mitochondrial DNA and gastrointestinal motor and sensory functions in health and functional gastrointestinal disorders

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Mitochondrial DNA and gastrointestinal motor and sensory functions in health and functional gastrointestinal disorders. Am J Physiol Gastrointest Liver Physiol 296: G510–G516, 2009. First published January 15, 2009; doi:10.1152/ajpgi.90650.2008—Nerve, muscle, and inflammatory cells involved in gastrointestinal (GI) function have high-energy requirements and are affected in mitochondrial disorders. Familial aggregation of irritable bowel syndrome (IBS) frequently involves mothers and their children. Since mitochondrial DNA (mtDNA) is maternally inherited, mtDNA single nucleotide polymorphisms (SNPs) could confer risk to the development of IBS. The mtDNA SNPs, 16519C>T and 3010G>A, are associated with migraine and childhood cyclic vomiting syndrome. Our hypothesis is that these mtDNA SNPs are associated with functional GI disorders (FGIDs) and GI functions. The mt genome was first tested for the 7028C polymorphism (defining haplogroup H) in 699 patients or controls, and those with 7028C were further genotyped at 16519 and 3010. Phenotypes were based on symptoms (validated questionnaires and criteria) and GI physiology using validated motor and sensory studies. Constipation-predominant IBS and alternating constipation and diarrhea IBS are less prevalent in individuals with the 7028C mtDNA polymorphism than in individuals with 7028T. Conversely, 7028C is associated with higher maximum tolerated volume (lower satiation) compared with 7028T. Among those with 7028C, nonspecific abdominal pain (chronic abdominal pain or dyspepsia) was significantly associated with 3010A compared with 3010G (odds ratio 3.3, P = 0.02), and slower gastric emptying was statistically associated with 3010G. There were no significant associations of mtDNA genotypes tested and stomach volumes, small bowel or colonic transit, rectal compliance, and motor or sensory functions. Thus variation in mtDNA may be associated with satiation, gastric emptying, and possibly pain; further studies of mtDNA in appetite and sensory studies. Constipation-predominant IBS and alternating constipation and diarrhea IBS are less prevalent in individuals with the 7028C mtDNA polymorphism than in individuals with 7028T. Conversely, 7028C is associated with higher maximum tolerated volume (lower satiation) compared with 7028T. Among those with 7028C, nonspecific abdominal pain (chronic abdominal pain or dyspepsia) was significantly associated with 3010A compared with 3010G (odds ratio 3.3, P = 0.02), and slower gastric emptying was statistically associated with 3010G. There were no significant associations of mtDNA genotypes tested and stomach volumes, small bowel or colonic transit, rectal compliance, and motor or sensory functions. Thus variation in mtDNA may be associated with satiation, gastric emptying, and possibly pain; further studies of mtDNA in appetite and sensory functions.

Complex multifactorial conditions, usually influenced by multiple genetic and environmental factors, are the cause of the vast majority of human disease. These conditions are becoming better understood as (nuclear) genetic polymorphisms that confer an increased risk toward disease pathogenesis are being identified.

There is evidence of familial aggregation of irritable bowel syndrome (IBS), which typically involves mothers and their children (26, 34). In a recent study of familial aggregation (34), there was a significant odds ratio (OR) for mothers and sisters of IBS probands to be affected with IBS (ORs 3.4 and 3.1, respectively). Studies exploring the genetic epidemiology of functional gastrointestinal disorders (FGID) have generally addressed the potential association with single mechanisms such as receptors, transporters, and translation or transduction mechanisms. To date, there is no definite evidence that any single genetic defect is associated with FGID. For example, in the most frequently reported association of genetic variation in IBS, a meta-analysis of several studies on 5-HTTLPR did not reveal a significant association with patients with IBS of Asian or European ethnicity relative to healthy people from the same ethnic groups (39). On the other hand, there is an association between GNB3 polymorphisms and functional dyspepsia (6, 23, 36, 40). Further studies of the association of common genetic polymorphisms with FGID are warranted.

The nerve, muscle, and inflammatory cells that may be involved in the mechanisms underlying the development of FGIDs (7) have high-energy requirements and are frequently affected in mitochondrial disorders. Mitochondria are cytoplasmic organelles that produce the bulk of the ATP for cellular energy needs. Mitochondrial proteins are encoded on both the nuclear DNA (chromosomes) as well as the 16-kilobase mitochondrial DNA (mtDNA). Thus sequence variants (polymorphisms) that adversely affect energy metabolism and predispose to disease pathogenesis theoretically could be on either or both of those genomes (25, 38).

Although the mitochondrial genome is small, at high copy number it constitutes a substantial fraction of the total cellular mass of DNA and has a very high polymorphic density (35). Thus mtDNA sequence variation is likely to affect an individual’s risk toward the development of some multifactorial conditions in a manner analogous to nuclear DNA polymorphisms (33).

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The cytoplasmically located mtDNA generally is derived solely from the ova (“maternal inheritance”) without recombination, and individuals related through women carry an identical mtDNA sequence in the absence of a recent mutation. It is conceivable that IBS, which has a significant OR to aggregate in mothers and sisters (34), may be associated with maternal inheritance of mtDNA sequence variants. Although nuclear DNA encodes 3,000 proteins involved in mitochondrial functions such as energy generation or transfer, the respiratory enzyme chain cannot function without the mtDNA-encoded subunits, and they cannot be translated without the 16S-rRNA.

Patients with mtDNA disorders frequently suffer from symptoms that overlap with FGIDs (5, 43). Cyclic vomiting syndrome (CVS) is a functional disorder that generally demonstrates maternal inheritance (2); mtDNA sequence variants have been reported in CVS (3, 24, 42, 44). In a previous study by Boles et al. (2), two common mtDNA single nucleotide polymorphisms (SNPs), 16519C (cytosine)→T (thymine) and 3010G (guanine)→A (adenine), were found to be associated with CVS and migraine among patients with the mtDNA SNP 7028C (haplogroup H).

Given the important role of mitochondria in neuromuscular function and inflammation and the possible role of these mechanisms in FGID (7), we explored the hypothesis that these mtDNA SNPs are associated with IBS, functional constipation, functional diarrhea, chronic abdominal pain, and functional dyspepsia. To explore this hypothesis, we sought the presence of associations between the three common mtDNA SNPs with symptom phenotype of FGIDs and gastrointestinal motor and sensory functions.

MATERIALS AND METHODS

Overall Design

We assessed symptom phenotype using consensus criteria with validated questionnaires that assessed gastrointestinal symptoms (37) in all participants [which included an assessment of somatic symptoms in a majority (63%) of the participants] and gastrointestinal function using validated motor and sensory studies. These were the satiation volume (maximum tolerated volume) and symptoms with a nutrient drink test (n = 116), gastrointestinal (n = 268) and colonic (n = 172) transit of solid food, residue by dual isotope scintigraphy, gastric volume (fasting and postmeal accommodation) by 99mTc-SPECT (n = 228), and rectal compliance and sensation by barostat (n = 116).

Participants

This study assessed 466 patients with FGIDs (Rome II positive, 19 with chronic abdominal pain, 175 with diarrhea-predominant IBS (IBS-D) or functional diarrhea, 155 with constipation-predominant IBS (IBS-C) or functional constipation, 84 with IBS-mixed or alternating (IBS-Alt), and 33 with dyspepsia) and 233 healthy volunteers recruited to studies of symptom phenotype and genotype from 2000–2007 (1, 6, 11, 27). All participants were residents of the region within 150 miles of Rochester, Minnesota. Participants had been recruited for the original studies (1, 6, 11, 27) by means of letters or public advertisements and had signed informed consent for the respective studies. The demographics of the patients and controls were similar (Supplemental Table S1). Supplemental material for this article is available online at the American Journal of Physiology Gastrointestinal and Liver Physiology website.

The inclusion criteria and characteristics of each patient group appear in the original studies; all patients fulfilled Rome II criteria. For example, the group with chronic (functional) abdominal pain had abdominal pain of at least 12 wk duration (not necessarily consecutive) in the absence of bowel dysfunction to differentiate from IBS. In addition, functional dyspepsia patients were identified by upper abdominal pain and discomfort related to food ingestion (11). All individual studies (1, 6, 11, 27) had been conducted with IRB approval. Use of the database from which this analysis was conducted was also reviewed and approved by the Mayo Clinic Institutional Review Board, and all participants had given permission for research studies on the basis of their medical records and DNA samples.

The validated bowel symptom questionnaire, review of the electronic medical record (S. McKinzie), or direct physician interview and examination (M. Camilleri) were used to characterize the subtype of FGID. The physiological measurements have been used extensively to characterize motor and sensory functions in patients with FGID and to document the effects of pharmacological agents on these functions in health and disease states.

Satiation by the Nutrient Drink Test

A standardized Ensure (1 kcal/ml, 11% fat, 73% carbohydrate and 16% protein) drink test (12) was used to measure satiation and postprandial symptoms of nausea, bloating, and pain 30 min after the meal in 116 participants.

Gastrointestinal and Colonic Transit by Scintigraphy

An adaptation of our established combined scintigraphic method was used and provided measurements of gastric (n = 268), small bowel (n = 219), and colonic transit (n = 172) over 48 h. In 98 participants, gastric emptying was measured by scintigraphy using the same radiolabeled meal, and scans were obtained over the first 4 h. The data were generally acquired in single center pharmacodynamic studies, the genetic association studies detailed above (1, 11, 27), or pharmacogenetics studies of the pharmacodynamic response to the drugs alosetron (8) or clonidine (9). The method, endpoints, data analysis, and performance characteristics of the test are described elsewhere (15). The primary transit endpoints for the association studies were the gastric emptying t1/2 and colonic geometric center at 24 h (GC24).

Gastric Volume by 99mTc-SPECT

Gastric volume was measured in 228 participants using a SPECT method developed and validated in our laboratory (4). We measured the gastric volume during fasting and after 300 ml of Ensure (300 kcal; Ross Laboratories, Abbott Park, IL). After intravenous 99mTc-sodium pertechnetate (0.12 mCi/kg), which is taken up by the gastric mucosa, the camera (SMV-GE, Fairfield, CT) rotates around the thorax and abdomen with the participant supine. The stomach was identified in the transaxial images and separated from background with a semiautomated segmentation algorithm using the AVW 3.0 (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN) image processing libraries. The primary endpoints were fasting and postprandial gastric volume.

Rectal Compliance and Sensation by Barostat

These studies were conducted in 112 subjects (87 patients with IBS and 25 healthy controls) who presented after bowel preparation (Fleet phosphate enema, self administered at least 1 h before reporting to the center) and an overnight fast. The studies were conducted as described in detail elsewhere (10) using a polyethylene bag (MUI Scientific, Mississauga, Ontario, Canada) inserted into the rectum so that the middle of the balloon was about 10 cm from the anal verge; subjects were in a semiprone position, and the foot end of the bed was elevated 15 degrees and the catheter connected to a barostat (G & J Electronics,
Toronto, Ontario, Canada). The baseline operating pressure was defined as 2 mmHg above the minimal distension pressure at which respiratory excursions were clearly recorded from the barostat tracing. An initial “conditioning” distension of the rectum was performed with pressure increased from 0 to 20 mmHg in steps of 4 mmHg for 15 s per step (22).

Rectal sensory thresholds were measured by ramp inflation, starting at 0 mmHg and increasing in steps of 4 mmHg for 1 min per step to a maximum of 60 mmHg. Thresholds for first sensation, gas, urgency, and pain were indicated by the subjects pressing a button at the distension pressure at which sensations were perceived. Ramp inflation was terminated when the subjects reported the first sensation of pain. Following this procedure, the bag was deflated to the baseline operating pressure and the subjects were allowed to rest for 10 min.

Rectal sensory ratings for gas, urgency, and pain were measured using separate 100-mm visual analog scales during phasic distensions of 12, 24, 30, and 36 mmHg above baseline operating pressure applied once in random order. The order was provided by the study statistician (A. Zinsmeister). Each distention was maintained for 60 s with an interstimulus interval of 2 min, during which time the balloon was deflated to the baseline operating pressure. The methods and analysis have been described in detail elsewhere (14).

**mtDNA Genotyping Methods**

DNA was isolated from blood by standard methods (6, 11, 27) in 699 of the 701 participants. The mtDNA haplogroups denote sets of matrilineal ancestry tens of thousands of years old. The presence or absence of the 7028C polymorphism that defines haplogroup H was determined by PCR/restriction fragment length polymorphism analysis following Alu digestion, with primer sequences (forward, TTTCGGTCAACCTGAAGTTA; reverse, AGCGAACGGTCTC-CAAATCAT). The West Eurasian haplogroup H is well suited for genetic association studies because of the relative lack of intragroup sequence variability and high prevalence. As in the previous study from the Boles laboratory (2), limiting the present study to subjects with haplogroup H substantially decreases background sequence variability and correspondingly increases statistical power. It is also practical because haplogroup H is the most common among European-derived populations, including prevalence rates of about 45% in the native population of Germanic countries and in about 33% of North Americans of apparent European ancestry (41). 16519T and 3010A are found in individuals with a multitude of haplogroups and within all major races. In fact, among Americans of apparent European origin, 16519T is designated herein as the “polymorphism,” despite 16519C being the reference nucleotide (33), because 16519T is both the ancestral nucleotide as well as the most common nucleotide seen in all major human races.

**Statistical Analysis**

The statistical analyses were structured in three parts. First, the associations with overall haplogroup status (7028C = haplogroup H vs. 7028T = all non-H haplogroups) were assessed; second, within the H haplogroup, the associations with 3010G vs. 3010A and separately 16519C vs. 16519T were evaluated. mtDNA differs in many substantial ways from nuclear DNA. For example, the polymorphisms in mtDNA are in complete linkage disequilibrium because mtDNA never recombines. Moreover, the 16519 locus is in the region that controls replication for the entire mtDNA, including 3010. Thus, third, we explored associations of symptom phenotype and gastrointestinal functions with the combinations of different genotypes at the 16519 locus and the different genotypes at the 3010 locus, that is, GC (e.g., GC = 3% G plus 16519C), GT, AC, and AT, among participants within haplogroup H.

**Symptom phenotype.** The overall univariate association of genotype with symptom phenotype was assessed using contingency table analyses ($\chi^2$ test), combining all FGIDs into one group and separately using the individual FGID subtypes. ORs (95% confidence intervals) for each symptom phenotype (compared to healthy controls) and the mtDNA genotypes (e.g., 7028C relative to 7028T, 3010G relative to 3010A, and 16519T relative to 16519C) were estimated using multiple logistic regression, adjusting for age and sex. Because of the small number of subjects in the AT combination, the multiple logistic regression model predicting individual symptom phenotypes was not examined for the genotype 3010A and 16519T combination.

**Gastrointestinal motor function.** The association with gastrointestinal motor function was assessed using analysis of covariance (ANCOVA), adjusting for age, sex, and body mass index. These analyses were done using all subjects, with physiology data to examine these associations separately for gastric emptying, small bowel transit, colonic transit, nutrient drink test challenges (maximum tolerated volume and aggregate symptom score 30 min post satiation), and gastric volumes as measured by SPECT.

**Rectal sensation.** The associations with each rectal sensation threshold type (gas, urgency, and pain) were assessed using the log-rank test for specific pairs (e.g., 7028C vs. T, 3010G vs. A, and 16519C vs. T, 3010G vs. A, and 16519T vs. 7028T).

**Table 1. Demographic, somatic, motor and satiation data**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>n = 399</th>
<th>n = 300</th>
<th>n = 190</th>
<th>n = 110</th>
<th>n = 210</th>
<th>n = 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>Non-H Haplogroup</td>
<td>27.3±0.3</td>
<td>27.4±0.3</td>
<td>27.4±0.4</td>
<td>27.3±0.5</td>
<td>27.3±0.4</td>
<td>27.5±0.6</td>
</tr>
<tr>
<td>Age, yr</td>
<td>H Haplogroup</td>
<td>41.4±0.7</td>
<td>43.8±0.8</td>
<td>42.7±1.0</td>
<td>45.6±1.4</td>
<td>44.7±1.0</td>
<td>41.8±1.4</td>
</tr>
<tr>
<td>Somatic symptom score</td>
<td>0.66±0.04</td>
<td>0.57±0.04</td>
<td>0.55±0.05</td>
<td>0.60±0.06</td>
<td>0.58±0.05</td>
<td>0.53±0.06</td>
<td></td>
</tr>
<tr>
<td>% Stomach emptied at 120 min</td>
<td>52.1±1.4</td>
<td>52.1±1.8</td>
<td>48.1±1.9</td>
<td>58.3±3.2</td>
<td>54.1±2.2</td>
<td>46.9±2.52</td>
<td></td>
</tr>
<tr>
<td>% Colonic filling at 6 h</td>
<td>27.2±2.8</td>
<td>22.5±3.3</td>
<td>28.8±4.6</td>
<td>13.5±4.1</td>
<td>18.9±3.8</td>
<td>31.0±6.2</td>
<td></td>
</tr>
<tr>
<td>Colon GC at 24 h</td>
<td>2.45±0.1</td>
<td>2.80±0.14</td>
<td>2.72±0.15</td>
<td>2.92±0.28</td>
<td>2.81±0.18</td>
<td>2.77±0.22</td>
<td></td>
</tr>
<tr>
<td>Max tolerated volume, ml</td>
<td>1021±36</td>
<td>1181±50</td>
<td>1221±63</td>
<td>1096±78</td>
<td>1154±68</td>
<td>1224±72</td>
<td></td>
</tr>
<tr>
<td>Aggregate symptom score</td>
<td>171.9±8.3</td>
<td>197.3±14.1</td>
<td>201.0±15.4</td>
<td>189.2±30.6</td>
<td>204.6±18.1</td>
<td>185.8±22.9</td>
<td></td>
</tr>
<tr>
<td>Fasting gastric volume, ml</td>
<td>232.1±7.1</td>
<td>245.5±8.5</td>
<td>244.7±11.4</td>
<td>246.9±12.6</td>
<td>240.2±8.6</td>
<td>258.6±20.2</td>
<td></td>
</tr>
<tr>
<td>ΔPP-fasting gastric vol, ml</td>
<td>508.7±8.4</td>
<td>506.9±9.1</td>
<td>513.4±10.7</td>
<td>496.8±16.4</td>
<td>505.5±11.7</td>
<td>510.3±13.2</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index. PP, postprandial.
16519T vs. C) and summarized as median (% censored) on the basis of the Kaplan-Meier product limit method. The association between colonic sensation visual analog scale ratings scores and genotype was based on repeated-measures ANCOVA (the repeated factor being the multiple pressure distension levels) and the Wilcoxon rank sum test at specific distension levels, separately for gas, urgency, and pain sensation types.

The aim in these hypotheses-generating analyses was to explore potential associations that would warrant further study, and thus no adjustment in \( \alpha \) level for multiple tests was made. In particular, \( P \) values between 0.05 and 0.1 were considered suggestive of potential associations that might deserve further study with larger numbers of subjects.

RESULTS

Haplogrouping of Study Participants

In this predominantly Caucasian cohort, 42.9% carried the 7028C SNP (haplogroup H). As expected, the 16519 polymorphism was associated with the 3010 polymorphism (\( P < 0.001 \)).

Association of mtDNA Genotype and Symptom Phenotype

Table 1 shows a summary of demographic, somatic symptom scores and physiological data. In Table 2, the proportions of FGID phenotypes in subjects with 7028C and 7028T are reported. Within subjects with 7028C (haplogroup H), data for 300 participants are subdivided further according to 3010 and 16519 genotype.

A somewhat lower odds for any FGID (compared to healthy controls) in 7028C (haplogroup H, relative to 7028T) was observed OR (95% confidence interval) = 0.7 (0.5, 0.96), \( P = 0.03 \), with, specifically, a lower odds for IBS-C [OR = 0.5 (0.3, 0.8), \( P = 0.006 \)] and a somewhat lower OR for IBS-Alt [OR = 0.6 (0.3, 0.96), \( P = 0.035 \)], as shown in Fig. 1.

An increased odds for any FGID (compared to healthy controls) was observed (Supplemental Table S2) in 3010A [relative to G, OR = 1.6 (0.9, 2.7), \( P = 0.11 \)]. A univariate association of 3010A (vs. G) with individual phenotype group \( P = 0.09 \) was observed (Table 3) and, specifically, an increased odds for chronic abdominal pain [OR = 4.9 (0.9, 27.), \( P = 0.07 \)], adjusted for age and sex. If one were to consider chronic abdominal pain and dyspepsia as a combined, nonspecific abdominal pain group, there was an increased odds for this condition (compared to controls) in the 3010A genotype [relative to the 3010G genotype, OR = 2.5 (0.95, 6.7), \( P = 0.06 \)].

No significant phenotypic associations were detected for 16519C vs. T, for any genotype and somatic symptom scores. For example, the OR for any FGID compared with healthy controls in the 16519C genotype relative to the T genotype was 1.3 (0.7, 2.2), \( P = 0.38 \).

Association of Haplogroup H with Gastrointestinal Functions

There were no significant associations for the presence of 7028C (vs. 7028T) with any other motor functions (gastrointestinal transit, specifically colonic filling at 6 h, GC at 24 h, GC at 48 h) or rectal compliance, aggregate symptom score after nutrient drink test, fasting volume (SPECT), and delta volume (fasting minus fed, Table 2). In contrast, there was a significant association (\( P = 0.037 \)) of 7028 status with satiation (the maximum tolerated volume of Ensure ingested during the nutrient drink test, Table 2), with higher volumes observed with 7028C overall. There was also a significant association between gastric emptying at 120 min and 3010 (\( P = 0.043 \)), with slower emptying in the 3010G genotype (vs. 3010A).

There were no significant associations detected for rectal compliance (\( P = 0.09 \)), sensation thresholds, or sensation ratings in people carrying 7028C vs. 7028T (Supplemental Table S1). However, although the overall sensation of gas ratings was not significant (\( P = 0.156 \)), sensation ratings for gas at 24 and 30 mmHg distension were lower with 7028C vs. 7028T (\( P = 0.032 \) and 0.031, respectively). Similarly, an

Table 2. Proportion (%) of FGID phenotypes in haplogroup H and combined non-H haplogroups

<table>
<thead>
<tr>
<th>Haplogroup</th>
<th>N</th>
<th>% FGID</th>
<th>% IBS-Alt</th>
<th>% IBS-C</th>
<th>% IBS-D</th>
<th>% FD</th>
<th>% CAP</th>
<th>% GE at 120 min</th>
<th>MTV, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>All non-H haplogroups (7028T)</td>
<td>399</td>
<td>69</td>
<td>14</td>
<td>25</td>
<td>23</td>
<td>4</td>
<td>3</td>
<td>52±1</td>
<td>1021±36</td>
</tr>
<tr>
<td>Haplogroup H (7028C)</td>
<td>300</td>
<td>64</td>
<td>10</td>
<td>19*</td>
<td>27</td>
<td>5</td>
<td>3</td>
<td>52±2</td>
<td>1181±50*</td>
</tr>
<tr>
<td>3010G genotype</td>
<td>190</td>
<td>61</td>
<td>10</td>
<td>21</td>
<td>25</td>
<td>4</td>
<td>1</td>
<td>48±2**</td>
<td>1221±63</td>
</tr>
<tr>
<td>3010A genotype</td>
<td>110</td>
<td>69</td>
<td>10</td>
<td>15</td>
<td>31</td>
<td>8</td>
<td>5</td>
<td>58±3</td>
<td>1096±78</td>
</tr>
<tr>
<td>16519C genotype</td>
<td>210</td>
<td>65</td>
<td>10</td>
<td>19</td>
<td>27</td>
<td>7</td>
<td>2</td>
<td>54±2</td>
<td>1154±68</td>
</tr>
<tr>
<td>16519T genotype</td>
<td>90</td>
<td>61</td>
<td>11</td>
<td>18</td>
<td>29</td>
<td>2</td>
<td>1</td>
<td>47±3</td>
<td>1224±72</td>
</tr>
</tbody>
</table>

Applicable values are means ± SE. Within the H haplogroup, data for 300 participants are subdivided further according to 3010 and 16519 genotypes or proportion (% of functional gastrointestinal disorder (FGID) phenotypes within each haplogroup or genotype. * \( P < 0.05 \) vs. all non-H haplogroups. ** \( P = 0.04 \) vs. G genotype. IBS, irritable bowel syndrome; Alt, alternating ("mixed"); C, constipation; D, diarrhea; FD, functional dyspepsia; CAP, chronic abdominal pain; % GE, percentage of meal emptied from the stomach at 120 min; MTV, maximum tolerated volume.
overall association of the four genotypes (within haplogroup H subjects) with the threshold sensation for gas was not significant ($P = 0.101$), and the repeated-measures analysis of variance for the sensory rating scores did not detect any significant associations (Supplemental Table S3).

**Association of Combinations of Different 3010 and 16519 Genotypes with Symptom Phenotype and Gastrointestinal Function in People with Haplogroup H**

Table 4 shows the distribution of FGID symptom phenotypes in different genotype combinations. Whereas univariate analysis (contingency table) indicated no overall significant association of genotype (7028C vs. T) with individual phenotype, $P = 0.16$, within haplotype H (with 3 out of 30 cells having values of zero), a Montecarlo “exact test” also indicated no overall association of genotype combination with individual phenotype $P = 0.35$. In addition, no associations with gastrointestinal functions or somatic scores (Table 4) were detected. Comparisons with the AT genotype combination are constrained by the small number ($n = 5$) with this combination of genotypes.

**DISCUSSION**

The present study has shown that haplogroup H (7028C) is associated with decreased odds for IBS-C and IBS-Alt, relative to all other haplogroups (7028T). The haplogroup H also was associated with increased satiation volume. Within haplogroup H, decreased gastric emptying at 120 min was found with the 3010G genotype (relative to the 3010A genotype). The significance of these observations is unclear and requires replication in an independent cohort of patients. A post hoc analysis suggested an association between 3010A genotype and a nonspecific abdominal pain group created by combining chronic abdominal pain and dyspepsia. This also requires confirmation in a separate cohort. In particular, patients with dyspepsia will need to be subclassified into those with epigastric pain syndrome rather than postprandial distress syndrome. Interestingly, it is known that accelerated gastric emptying is one of the factors that contribute to development of dyspepsia (16). In our participants, both pain and relatively faster gastric emptying were associated with 3010A. However, there was lower colonic filling at 6 h in the 3010A group, suggesting that, in contrast to the relatively faster gastric emptying, there may be slower small bowel transit. This cannot be explained by slower colonic transit (which might conceivably retard small bowel emptying into the colon). The differences for colonic filling in the 3010A vs. 3010G groups are not, however, statistically significant. We have no explanation for an apparent discrepancy between accelerated gastric emptying and the somewhat slower small bowel transit. Further studies would be needed to show whether small bowel transit is significantly different according to the 3010 genotype. Unlike what was reported in migraine (2), in the present study the effects of 3010A vs. G are independent of the 16519 genotype.

The increased maximum tolerated volume associated with haplogroup H overall may also increase upper gastrointestinal symptoms. Epidemiological data from patients with obesity (which is associated with increased maximum tolerated calorie intake, Ref. 18) in a United States community showed a significant increase in the reporting of abdominal pain (17). Furthermore, the present association of 3010A with nonspecific abdominal pain is consistent with the Boles group’s need to be subclassified into those with epigastric pain group created by combining chronic abdominal pain and dyspepsia. This also requires confirmation in a separate cohort. In particular, patients with dyspepsia will need to be subclassified into those with epigastric pain syndrome rather than postprandial distress syndrome. Interestingly, it is known that accelerated gastric emptying is one of the factors that contribute to development of dyspepsia (16). In our participants, both pain and relatively faster gastric emptying were associated with 3010A. However, there was lower colonic filling at 6 h in the 3010A group, suggesting that, in contrast to the relatively faster gastric emptying, there may be slower small bowel transit. This cannot be explained by slower colonic transit (which might conceivably retard small bowel emptying into the colon). The differences for colonic filling in the 3010A vs. 3010G groups are not, however, statistically significant. We have no explanation for an apparent discrepancy between accelerated gastric emptying and the somewhat slower small bowel transit. Further studies would be needed to show whether small bowel transit is significantly different according to the 3010 genotype. Unlike what was reported in migraine (2), in the present study the effects of 3010A vs. G are independent of the 16519 genotype.

The increased maximum tolerated volume associated with haplogroup H overall may also increase upper gastrointestinal symptoms. Epidemiological data from patients with obesity (which is associated with increased maximum tolerated calorie intake, Ref. 18) in a United States community showed a significant increase in the reporting of abdominal pain (17). Furthermore, the present association of 3010A with nonspecific abdominal pain is consistent with the Boles group’s

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**Table 3. Distribution (%) of FGID on the basis of symptoms in H haplogroup-based genotype combinations at positions 3010 and 16519**

<table>
<thead>
<tr>
<th>Mitochondrial DNA</th>
<th>Total n (DNA type)</th>
<th>IBS-Alt %</th>
<th>Chronic Abdominal Pain %</th>
<th>Constipation or IBS-C %</th>
<th>Diarrhea or IBS-D %</th>
<th>Dyspepsia %</th>
<th>Health %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-H haplogroup</td>
<td>399</td>
<td>13.53</td>
<td>3.01</td>
<td>24.81</td>
<td>23.31</td>
<td>4.26</td>
<td>31.08</td>
</tr>
<tr>
<td>GC</td>
<td>105</td>
<td>9.32</td>
<td>0.95</td>
<td>21.90</td>
<td>23.81</td>
<td>4.76</td>
<td>39.05</td>
</tr>
<tr>
<td>GT</td>
<td>85</td>
<td>10.59</td>
<td>1.18</td>
<td>18.82</td>
<td>27.06</td>
<td>2.35</td>
<td>40</td>
</tr>
<tr>
<td>AC</td>
<td>105</td>
<td>9.32</td>
<td>4.76</td>
<td>16.19</td>
<td>29.52</td>
<td>8.57</td>
<td>31.43</td>
</tr>
<tr>
<td>AT</td>
<td>5</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>60</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

Chi-square $P = 0.16$ for haplotype H vs. non-H.

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**Table 4. Demographic and motor physiological data by 3010 (G or A) and 16519 (C or T) genotype combinations in those with haplotype H**

<table>
<thead>
<tr>
<th>Group</th>
<th>GC n = 105</th>
<th>GT n = 85</th>
<th>AC n = 105</th>
<th>AT n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, F (%)</td>
<td>81</td>
<td>88</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.2 ± 0.5</td>
<td>27.7 ± 0.6</td>
<td>27.4 ± 0.5</td>
<td>24.2 ± 1.3</td>
</tr>
<tr>
<td>Age, yr</td>
<td>43.7 ± 1.5</td>
<td>41.5 ± 1.4</td>
<td>45.6 ± 1.5</td>
<td>46.2 ± 1.7</td>
</tr>
<tr>
<td>Somatic symptom score</td>
<td>0.56 ± 0.07</td>
<td>0.54 ± 0.07</td>
<td>0.60 ± 0.07</td>
<td>0.34 ± 0.28</td>
</tr>
<tr>
<td>% Stomach emptied at 120 min</td>
<td>49.6 ± 0.7</td>
<td>46.1 ± 0.6</td>
<td>58.2 ± 3.3</td>
<td>55.0 ± 1.1</td>
</tr>
<tr>
<td>% Colon filling at 6 h</td>
<td>25.4 ± 0.4</td>
<td>32.9 ± 0.7</td>
<td>13.3 ± 4.3</td>
<td>16.0 ± 1.5</td>
</tr>
<tr>
<td>Colon GC at 24 h</td>
<td>2.66 ± 0.21</td>
<td>2.78 ± 0.22</td>
<td>2.95 ± 0.29</td>
<td>2.69 ± 0.02</td>
</tr>
<tr>
<td>Max⁰ tolerated volume, ml</td>
<td>1208 ± 106</td>
<td>1232 ± 76</td>
<td>1096 ± 85</td>
<td>1105</td>
</tr>
<tr>
<td>Aggregate symptom score</td>
<td>216.0 ± 17.7</td>
<td>188.1 ± 20.4</td>
<td>192.3 ± 33.1</td>
<td>152</td>
</tr>
<tr>
<td>Fasting gastric volume, ml</td>
<td>234.8 ± 11.5</td>
<td>257.2 ± 21.4</td>
<td>245.2 ± 12.9</td>
<td>277.3 ± 75.8</td>
</tr>
<tr>
<td>ΔPP-fasting gastric vol, ml</td>
<td>516.3 ± 15.8</td>
<td>509.7 ± 17.3</td>
<td>495.7 ± 17.3</td>
<td>517.7 ± 31.3</td>
</tr>
</tbody>
</table>

Applicable values are means ± SE. Data without SE refer to information where the $n = 1$. 

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previously reported association of this SNP with both migraine and CVS, conditions in which abdominal pain/discomfort is common. Although prior work has reported an association between mitochondrial dysfunction (as measured by ATP production rate in biopsied muscle) and the presence of somatic symptoms in general (19, 20), in the present study, we found no association between the three mtDNA SNPs studied and the overall somatic symptom score. Further research is needed to better understand these findings.

Although haplogroup H is defined by the 7028C mtDNA SNP, it is unknown which mtDNA polymorphism(s) actually confers the functional consequence because haplogroup H is highly complex with multiple constituent subhaplogroupings. On the other hand, in our previous study, 16519T and 3010A were considered highly likely to confer the functional consequences in CVS because the full mtDNA genomic sequences in those subjects were available for analysis. This led to the present study to explore whether these SNPs are also factors associated with FGID. For the purpose of analysis of the FGID subgroups with altered bowel function in this study, the analysis pooled participants with functional diarrhea with IBS-D and those with functional (chronic) constipation with IBS-C. The rationale for this grouping is based on the increasing evidence that there is significant overlap of symptoms; that challenges the present paradigm that FGID represent multiple discreet entities (28) and the observation of transition among symptom subgroups, such as IBS-C and functional constipation, as well as IBS-D and functional diarrhea over a 12-yr period (21).

The 16519 SNP is located in the 1-kb noncoding mtDNA control region ("D-loop"), whereas the 3010 SNP is located in the 16S ribosomal RNA gene. Both polymorphisms are located in areas with relatively low sequence heterogeneity, have arisen multiple times in human evolution, are present in all major human races, and have been reported to likely have functional consequences (2, 33). 16519T is associated with diabetes and a poorer prognosis in individuals with pancreatic cancer (31), and a physiological effect of this polymorphism is also suggested by its complex effects on human exercise physiology (30). 3010A defines two East Asian subhaplogroups, J1 and D4, which are overrepresented in centenarians (32), but 3010A by itself has not previously been found to be statistically associated with human disease other than CVS and migraine (2). The present data suggest that 3010G may be associated with slower gastric emptying at 120 min compared with the 3010A genotype. It is unclear whether the relatively faster gastric emptying might explain the development of vomiting in CVS and migraine. Accelerated gastric emptying has been reported in CVS (18).

There are several points of caution in the interpretation of our study. First is the relatively small sample size for genotype to symptom phenotype associations. We chose to sacrifice a larger number of subjects for substantially greater genotypic homogeneity by limiting this study to subjects of haplogroup H. Moreover, the H vs. non-H assessment of associations had reasonable sample sizes to check for clinically meaningful associations. On the other hand, we have included unique and validated measures of gastrointestinal motor and sensory functions to explore possible hypotheses regarding the association between mtDNA and gastrointestinal functions. Thus the sample size in the study is generally appropriate for the study of associations between the mtDNA genotype and haplogroups (with their documented prevalence in the community) and the motor, satiation, and sensory functions. Because several other research groups are developing similar databases and capabilities, it is important to report our data and for others to test the hypotheses generated by our study and assess whether the data are replicated by independent groups studying different patient cohorts.

Second, the functional effects of the two SNPs at position 3010 and 16519 in a model cell or reporter system have not been demonstrated, and, therefore, even if there is an association, the mechanism whereby dysfunction or symptoms occur is unclear. It is also conceivable that 3010 and 16519 may serve simply as markers that are in linkage disequilibrium with an etiologically significant genetic variant, if such a relationship truly exists (e.g., between 3010 and altered gastric emptying).

Third, one should consider whether familial aggregation studies unequivocally support a component of maternal inheritance since an unequivocal maternal pattern of inheritance would support the potential role of mtDNA in the inherited component of IBS. It is worth noting, therefore, that, although the OR for female transmission in IBS with familial aggregation is significant, whereas that for males and brothers is not (34), the sample size for men in the previously published paper (34) was small (−18% of cohort), the confidence intervals wide (OR 4.2, confidence interval 0.8, 21.0), and a type II error cannot be excluded. Thus, familial aggregation studies do not exclude a nonmaternal inheritable component in IBS.

We conclude from this exploratory study that the Western Eurasian mtDNA haplogroup H (7028C genotype) is associated with decreased ORs for IBS-C and IBS-Alt. The H haplogroup may also influence satiation. Within haplogroup H, the common mtDNA polymorphism, 3010A, is associated with nonspecific abdominal pain and accelerated gastric emptying at 120 min. These disturbances of gastric function may be relevant in disorders of satiation (e.g., anorexia nervosa or obesity) and gastric emptying (e.g., gastroparesis and functional dyspepsia). Further studies are needed to clarify whether the effect of these polymorphisms may be dependent on the background haplogroup and to assess whether the significant associations with pain, emptying, and satiation result directly from functional effects of the polymorphisms on gastrointestinal motor and sensory functions. Investigation of the endophenotype (29) in the present study provides the first evidence that mtDNA variation influences gastric functions in health and FGID and that reported associations of mtDNA in functional syndromes such as CVS and migraine may have an identified physiological basis. Further studies of mtDNA in appetite regulation and larger numbers of patients with FGID are warranted.
FUNCTIONAL GASTROINTESTINAL DISORDERS AND mtDNA POLYMORPHISMS


