Mitochondrial DNA, Gastrointestinal Motor and Sensory Functions in Health and Functional Gastrointestinal Disorders

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ABSTRACT (248 words)

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 further genotyped at 16519 and 3010. Phenotypes were based on symptoms (validated questionnaires and criteria) and GI physiology using validated motor and sensory studies. IBS-C and IBS-Alt 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 to 7028T. Among those with 7028C, non-specific abdominal pain (chronic abdominal pain or dyspepsia) was significantly associated with 3010A compared to 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, motor or sensory functions. Thus, variation in mtDNA may be associated with satiation, gastric emptying and possibly pain; further studies of mtDNA in appetite regulation and larger numbers of patients with FGIDs are warranted.
**Key words:** 16519T, 3010A, 7028C, IBS, dyspepsia, mtDNA, haplogroup H, genotype, irritable bowel syndrome, IBS, constipation, dyspepsia, non-specific abdominal pain, gastric emptying, accommodation, satiation, somatic symptoms

**Abbreviations:**

- BMI: body mass index
- IBS: irritable bowel syndrome
- IBS-D: diarrhea-predominant irritable bowel syndrome
- IBS-C: constipation-predominant irritable bowel syndrome
- IBS-Alt: irritable bowel syndrome with alternating bowel function
- SSC: somatic symptom score
INTRODUCTION

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 for mothers and sisters of IBS probands to be affected with IBS (odds ratios 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-analyses of several studies on 5-HTTLPR did not reveal a significant association with IBS patients of Asian or European ethnicity relative to healthy people from the same ethnic groups (39). On the other hand, there is an association between GNβ3 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).

The cytoplasmic-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 odds ratio to aggregate in mothers and sisters (34), may be associated with maternal inheritance of mtDNA sequence variants. Although nuclear DNA encodes 3000 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., two common mtDNA single nucleotide polymorphisms (SNPs), 16519C>T and 3010G>A, were found to be associated with CVS and migraine among patients with the mtDNA SNP 7028C [haplogroup H (2)].

Given the important role of mitochondria in neuromuscular function, 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, MTV) and symptoms with a nutrient drink test (n=116), gastrointestinal (n=268) and colonic (n=172) transit of solid food and residue by dual isotope scintigraphy, gastric volume (fasting and post-meal accommodation) by $^{99m}$Tc-SPECT (n=228), and rectal compliance and sensation by barostat (n=116).

**Participants**

This study assessed 466 patients with FGIDs [Rome II positive; 19 chronic abdominal pain, 175 diarrhea-predominant IBS (IBS-D) or functional diarrhea, 155 constipation-predominant IBS (IBS-C) or functional constipation, 84 IBS-mixed or alternating (IBS-Alt), and 33 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
The demographics of the patients and controls were similar (Appendix Table A).

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 weeks duration (not necessarily consecutive) in the absence of bowel dysfunction in order to differentiate from IBS. In addition, functional dyspepsia patients were identified by upper abdominal pain and discomfort related to food ingestion (11). Use of the database from which this analysis was conducted was reviewed and approved by the Mayo Clinic Institutional Review Board, and all participants had given permission for research studies based on their medical records and DNA samples.

The validated bowel symptom questionnaire, review of the electronic medical record (SM), or direct physician interview and examination (MC) 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 minutes 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 hours. In 98 participants, gastric emptying was measured by scintigraphy using the same radiolabeled
meal and scans were obtained over the first 4 hours. 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 hours (GC24).

**Gastric Volume by 99mTc-SPECT**

Gastric volume was measured in 228 participants using a SPECT method developed and validated in our lab (4). We measured the gastric volume during fasting and post-300 ml Ensure® (300 kcal, Ross Labs, Abbott Park, IL). After intravenous 99mTc-sodium pertechnetate (0.12mCi/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 semi-automated 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 hour 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 in a semi-prone position and the foot end of the bed was elevated
15 degrees and the catheter connected to a barostat (G&J Electronics Inc., 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 seconds per step (22).

Rectal sensory thresholds were measured by ramp inflation, starting at 0 mmHg and increasing in steps of 4 mmHg for 1 minute 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 minutes.

Rectal sensory ratings for gas, urgency, and pain were measured using separate 100 mm visual analog scales (VAS) 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 (ARZ). Each distention was maintained for 60 seconds with an inter-stimulus interval of 2 minutes, 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 AluI digestion, with primer sequences (F TTTCGGTCAACCCTGAAGTTTA, and R
AGCGAAGGCTTCTCAAATCAT). The West Eurasian haplogroup H is well suited for genetic association studies due to the relative lack of intra-group 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, as 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 European origin, 16519T is actually slightly more common among non-haplogroup H individuals (13).

Participants with 7028C (haplogroup H) were tested for the 16519C>T and 3010G>A polymorphisms by PCR/restriction fragment length polymorphism (16519: HaeIII F GGATGACCCCTCAGATA, R CTTATTTAAGGGGAACGTG; 3010: BccI F CATGCTAAGACTTCACCA, R TCGTTGAACAAACGAACC). Genotypes were confirmed by random direct sequencing at Mayo Clinic’s DNA Sequencing Core Facility using Applied Biosystems BigDye® terminator v1.1 cycle sequencing chemistry and analyzed on Applied Biosystems 3730XL DNA Analyzer. 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 as 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, 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. Odds ratios (95% CIs) 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 gender. Due to 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.

**GI motor function**

The association with GI motor function was assessed using analysis of covariance (ANCOVA), adjusting for age, gender, and body mass index (BMI). 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 minutes post satiation), and gastric volumes as measured by SPECT.
Rectal sensation

The associations with each rectal sensation threshold type (gas, urgency and pain) was assessed using the log-rank test for specific pairs (e.g. 7028C vs. T, 3010G vs. A, and 16519T vs. C) and summarized as median (% censored) based on the Kaplan-Meier product limit method. The association between colonic sensation VAS 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 α 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%CI)] = 0.7(0.5, 0.96), p=0.03, with,
specifically, a lower odds for IBS-C \[OR=0.5 \text{ (0.3, 0.8)}, \ p=0.006\] and a somewhat lower odds ratio for IBS-alternating \[OR=0.6 \text{ (0.3,0.96), p=0.035}\], as shown in Figure 1.

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

No significant phenotypic associations were detected for 16519C versus T, for any genotype and somatic symptom scores. For example, the odds ratio for any FGID compared to healthy controls in the 16519C genotype relative to the T genotype was \text{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 CF@6hrs, GC@24hrs, GC@48hrs), 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 (\text{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 minutes and 3010 (\text{p=0.043}), with slower emptying in the 3010G genotype (vs. 3010A).

There were no significant associations detected for rectal compliance (\text{p=0.09}), sensation thresholds or sensation ratings in people carrying 7028C versus 7028T (Appendix Table A).
However, while 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 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 (Appendix Table C).

**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. While 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 minutes 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 hours 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 current study the effects of 3010A versus 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 (18)] in a U.S. 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 previously reported association of this SNP with both migraine and cyclic vomiting syndrome, conditions in which abdominal pain/discomfort is common. While 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 current 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.

While haplogroup H is defined by the 7028C mtDNA SNP, it is unknown which mtDNA polymorphism(s) actually confers the functional consequence, as haplogroup H is highly complex with multiple constituent sub-haplogroupings. On the other hand, in our previous study, 16519T and 3010A were considered highly likely to confer the functional consequences in cyclic vomiting syndrome, as the full mtDNA genomic sequences in those subjects were available for analysis. This led to the current 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 current paradigm that functional GI disorders 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 year period (21).

The 16519 SNP is located in the 1-kb non-coding mtDNA control region (“D-loop”), while 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 sub-haplogroups, J1 and D4, which are over-represented in centenarians (32), but 3010A by itself has not previously been found to be statistically associated with human disease other than cyclic vomiting syndrome and migraine (2). The present data suggest that 3010G may be associated with slower gastric emptying at 120 minutes compared to the 3010A genotype. It is unclear whether the relatively faster gastric emptying might explain the development of vomiting in cyclic vomiting syndrome and migraine. Accelerated gastric emptying has been reported in cyclic vomiting syndrome (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. As 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 odds ratios 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, CI 0.8, 21.0) and a type II error cannot be excluded. Thus, familial aggregation studies do not exclude a non-maternal inheritable component in IBS.

We conclude, from this exploratory study, that the Western Eurasian mtDNA haplogroup H (7028C genotype) is associated with decreased odds ratios for IBS-C and IBS-Alt. The H haplogroup may also influence satiation. Within haplogroup H, the common mtDNA polymorphism, 3010A, is associated with non-specific abdominal pain and accelerated gastric emptying at 120 minutes. 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 upon 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 current 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 cyclic vomiting syndrome and migraine may have an identified physiological basis. Further studies of mtDNA in appetite regulation and larger numbers of patients with FGID are warranted.
REFERENCES


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Table 1. Demographic, Somatic, Motor and Satiation Data (mean ± SEM)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Non-H Haplogroup n=399</th>
<th>H Haplogroup N=300</th>
<th>3010 G genotype n=190</th>
<th>3010 A genotype n=110</th>
<th>16519 C genotype n=210</th>
<th>16519 T genotype n=90</th>
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</thead>
<tbody>
<tr>
<td>Variable</td>
<td>BMI, kg/m²</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>
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<td>Age</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>
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<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>
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<td>% Stomach emptied @120 min</td>
<td>52.1 ± 1.4</td>
<td>52.1 ± 1.8</td>
<td>48.1 ± 1.9</td>
<td>58.0 ± 3.2</td>
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<td>% Colonic filling @ 6 hr</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>
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<td>Colon GC @ 24 hr</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>
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<td></td>
<td>Max⁰ tolerated volume, ML</td>
<td>1021 ± 36</td>
<td>1181 ± 50</td>
<td>1221 ± 63</td>
<td>1096 ± 78</td>
<td>1154 ± 68</td>
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<td></td>
<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>
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<td></td>
<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>
</tr>
<tr>
<td></td>
<td>Δ PP-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>
</tr>
</tbody>
</table>
Table 2. Proportion (%) of FGID Phenotypes in Haplogroup H and Combined Non-H Haplogroups (within the H haplogroup, data for 300 participants are subdivided further according to 3010 and 16519 genotypes; data are mean ± SEM or proportion (%) of FGID phenotypes within each haplogroup or genotype)

<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 @ 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>

A=alternating (“mixed”), C=constipation, D=diarrhea; * p<0.05 vs. all non-H haplogroups; ** p=0.04 vs. A genotype; FD=functional dyspepsia

% GE refers to percentage of meal emptied from the stomach at 120 minutes.
Table 3. Distribution (%) of Functional GI Disorders Based on Symptoms in H Haplogroup-Based Genotype Combinations at Position 3010 and 16519 (Chi-square p=0.16 for Haplotype H vs. non-H)

<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>9.52</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>9.52</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>
Table 4. Demographic and Motor Physiological Data by 3010 (G or A) and 16519 (C or T) Genotype Combinations in those with Haplotype H (mean ± SEM). Data without SEM refer to information where the n=1

<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>Gender, 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</td>
<td>43.7 ± 1.5</td>
<td>41.5 ± 1.4</td>
<td>45.6 ± 1.5</td>
<td>46.2 ± 6.7</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 @120 min</td>
<td>49.6 ± 2.7</td>
<td>46.1 ± 2.6</td>
<td>58.2 ± 3.3</td>
<td>55.0 ± 11.1</td>
</tr>
<tr>
<td>% Colonic filling @6 hr</td>
<td>25.4 ± 6.4</td>
<td>32.9 ± 6.7</td>
<td>13.3 ± 4.3</td>
<td>16.0 ± 15.5</td>
</tr>
<tr>
<td>Colon GC @ 24 hr</td>
<td>2.66 ± 0.21</td>
<td>2.78 ± 0.22</td>
<td>2.95 ± 0.29</td>
<td>2.69 ± 1.02</td>
</tr>
<tr>
<td>Maxm 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>Δ PP-fasting gastric vol, mL</td>
<td>516.3 ± 15.8</td>
<td>509.7 ± 14.2</td>
<td>495.7 ± 17.3</td>
<td>517.7 ± 31.3</td>
</tr>
</tbody>
</table>
FIGURE LEGEND

Figure 1  Odds ratio for specific phenotype versus healthy volunteer status in mtDNA haplogroup H (relative to non-H haplogroups)