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Genetic variation in endocannabinoid metabolism, gastrointestinal motility, and sensation

Michael Camilleri,1 Paula Carlson,1 Sanna McKinzie,1 April Grudell,1 Irene Busciglio,1 Duane Burton,1 Kari Baxter,1 Michael Ryks,1 and Alan R. Zinsmeister2

1Clinical Enteric Neuroscience Translational and Epidemiological Research and 2Department of Health Sciences Research, Division of Biostatistics, College of Medicine, Mayo Clinic, Rochester, Minnesota

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Camilleri M, Carlson P, McKinzie S, Grudell A, Busciglio I, Burton D, Baxter K, Ryks M, Zinsmeister AR. Genetic variation in endocannabinoid metabolism, gastrointestinal motility, and sensation. Am J Physiol Gastrointest Liver Physiol 294: G13–G19, 2008. First published October 25, 2007; doi:10.1152/ajpgi.00371.2007.—Cannabinoid agonist inhibits gastrointestinal motility. The endocannabinoid, anandamide, is inactivated by fatty acid amide hydrolase (FAAH). A single nucleotide polymorphism in the human FAAH gene (C385A) reduces FAAH expression. Our aim was to evaluate associations between FAAH genotype variation and symptom phenotype, gastric emptying and volume, colonic transit, and rectal sensation in patients with functional gastrointestinal disorders (FGID). 482 FGID patients (Rome II positive, 159 constipation disorders, 184 diarrhea disorders (D-IBS), 86 mixed bowel function (M-IBS), 20 chronic abdominal syndrome (IBS), functional dyspepsia, and chronic (functional) abdominal pain (CAP), 33 functional dyspepsia), and 252 healthy volunteers (HV) underwent questionnaires and studies of phenotype and genotype from 2000 to 2007: 250 gastric emptying, 210 fasting and postprandial gastric volume, 152 colonic transit, and 123 rectal sensation. All had FAAH genotype [CC vs. polymorphic (CA/AA)] determined by TaqMan. FAAH genotype distribution of FGID patients and HV did not deviate from Hardy-Weinberg equilibrium. There was a significant association of FAAH genotype with FGID phenotype (overall $\chi^2 = 2.11$, $P = 0.011$) and with specific individual phenotypes ($P = 0.048$). Thus FAAH CA/AA increases the odds (relative to HV) for D-IBS ($P = 0.008$), M-IBS ($P = 0.012$), and, possibly, CAP ($P = 0.055$). There was a significant association of FAAH CA/AA genotype with accelerated colonic transit in D-IBS ($P = 0.037$). There was no association of FAAH genotype with rectal sensation thresholds or ratings. The association of genetic variation in metabolism of endocannabinoids with symptom phenotype in D-IBS and M-IBS and with faster colonic transit in D-IBS supports the hypothesis that cannabinoids mechanisms may play a role in the control of colonic motility in humans and deserve further study.

rectal sensation; transit anandamide; fatty acid amide hydrolase

CANNABINOID (CB) receptors are located on cholinergic neurons in the brain stem, stomach, and colon. In mice, endocannabinoids acting on myenteric CB1 receptors tonically inhibit colonic propulsion (31). There is evidence of a direct role for cannabinoids in the modulation of motor activity in the human colon. Activation of CB1 receptors coupled to cholinergic motor neurons inhibits excitatory nerve transmission in human colonic circular muscle (21). We have shown that a nonspecific CB receptor agonist, dronabinol, inhibits gastric emptying and colonic motility in humans (16, 17). CB receptors are also involved in nociception (30, 32) and in mediating inflammation (26).

Endogenous cannabinoids (or endocannabinoids) include 2-arachidonyl glycero and anandamide. The latter is inactivated by a fatty acid amide hydrolase (FAAH) in vivo. Anandamide is a partial or full agonist of CB1 receptors. Endocannabinoids are synthesized in postsynaptic neurons and they are released into the synaptic cleft where they function as retrograde messengers (38), binding to the presynaptic CB1 receptor. This, in turn, acts on a range of effectors (e.g., adenyl cyclase, MAPK, and $K^+$ and $Ca^{2+}$ channels) via $G_{a,i}$ proteins. The inhibition of adenyl cyclase activity and the subsequent decrease in cAMP content lead to reduced activity of protein kinases with modulation of ion channels and reduced neurotransmitter release, e.g., acetyl choline. Thus a nonspecific cannabinoid agonist may inhibit contractile activity of the human gastrointestinal tract, and this was demonstrated with the retardation of gastric emptying (17) and the reduction of the tonic and phasic contractile responses of the colon to the ingestion of a meal in humans (16).

Cannabinoids have significant effects on visceral and somatic sensation. Their antinociceptive effects involve the brain, spinal cord, and peripheral sensory nerves (reviewed in Ref. 30). In acute pain models, anandamide, tetrahydrocannabinol, cannabidiol, and synthetic cannabinoids such as CP 55,940 and WIN 55,212-2 reduce pain induced by chemical, mechanical, and thermal stimuli (30). Similarly, anandamide and cannabinoid ligands are very effective against chronic pain of neuropathic or inflammatory origin in animals (30). These data suggest that CB1 receptors mediate, in part, the nociceptive response to pain in animal models of bowel inflammation (32).

Anandamide is also a ligand for TRPV1 receptors, with affinity lower than for CB1 receptors. This suggests that TRPV1 may also be involved in the analgesic effect of endogenous anandamide. Overall, cannabinoids influence motor, sensory, and immune functions. These functions have been proposed as mechanisms in the development of functional gastrointestinal disorders (FGID), including irritable bowel syndrome (IBS), functional dyspepsia, and chronic (functional) abdominal pain.

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A single nucleotide polymorphism (SNP) in the human-
FAAH gene (C385A, rs324420) is the only common SNP in the 
FAAH gene with a minor allele frequency >5%. In homozy-
gous AA form, the SNP converts a conserved proline residue in 
FAAH to threonine (P129T), reducing cellular expression of 
FAAH due to a posttranslational mechanism that is not pro-
tease-mediated degradation (11).

The rationale of this study was based on the potential for 
genetic variation in anandamide metabolism to influence the 
synaptic level of the endocannabinoid and, hence, the degree to 
which presynaptic modulation could influence motor or sen-
sory function, as well as the development of gastrointestinal 
symptoms. The aim of the study was to evaluate the association of 
FAAH C385A genotype with symptoms and physiological 
functions by comparing the odds of carrying the non-wild-type 
genotype and the occurrence of symptoms or abnormal motor 
or sensory functions in FGID patients compared with normal 
healthy volunteers.

MATERIALS AND METHODS

Participants. This study assessed 482 FGID patients [Rome II 
positive, 20 chronic abdominal pain (CAP), 184 functional diarrhea 
(D-IBS), 159 functional constipation (C-IBS), 86 mixed bowel function 
(M-IBS), and 33 dyspepsia] and 252 healthy volunteers recruited to 
studies of symptom phenotype and genotype from 2000 to 2007. 
All participants were recruited from the local population or were 
residents of the region within 150 miles of Rochester, MN. Particip-
ants had been recruited for the original studies (1, 7, 10, 24) by 
means of letters or public advertisement and had signed informed 
consent for the respective studies. The inclusion criteria and charac-
teristics of each patient group appear in the original studies; patients 
fulfilled Rome II criteria (15a). 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 dys-
function, to differentiate from IBS. In addition, functional dyspepsia 
patients were identified by upper abdominal pain and discomfort that 
was related to food ingestion (10). 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 medical records and their 
DNA samples.

All participants underwent bowel disease (including somatic symp-
tom) questionnaires, and 250 underwent gastric emptying by scintig-
raphy, 210 gastric volume measurements during fasting and postpran-
dially by 99mTc-SPECT, 152 colonic transit by scintigraphy, and 126 
rectal sensation studies. 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 func-
tions in patients with FGID and to document the effects of pharma-
cological agents on these functions in health and disease states.

Gastrointestinal and colonic transit by scintigraphy. An adaptation 
of our established scintigraphic method was used to measure gastro-
intestinal and colonic transit in 152 participants (5, 8, 14). Briefly, 
indium-111 (111In) adsorbed on activated charcoal particles was 
delivered to the colon by means of a methacrylate-coated, delayed-
release capsule administered by mouth. The capsule was ingested 
following an overnight fast. After the capsule emptied from the 
stomach (documented by its position relative to radiotrophic markers 
placed on the anterior iliac crest), a radiolabeled meal was ingested. 
In this meal, technetium-99m (99mTc) sulfur colloid was used to label 
two scrambled eggs that were eaten with one slice of whole wheat 
bread and one glass of whole milk (300 kcal total). This meal 
facilitated measurement of gastric and small bowel transit. Subjects ingested standardized meals for lunch and dinner at 4 and 8 h, 
respectively, after the radiolabeled meal. Abdominal scans were 
obtained every hour for the first 6 h (the first 4 h for the assessment of 
gastric emptying) and at 8, 24, and 48 h after ingestion of the 111In 
capsule. The performance characteristics of this test were summarized 
elsewhere (27).

Transit data analysis. A variable region of interest program was 
used to quantify the counts in the stomach and each of four colonic 
regions: ascending, transverse, descending, and combined sigmoid 
and rectum. These counts were corrected for isotope decay, tissue 
attenuation, and downscatter of 111In counts in the 99mTc window.

Gastric emptying half-time (t1/2) is a measure of the time for 50% 
of the radiolabeled meal (identifiable by radiolabeled tracer) to empty 
from the stomach. Overall colonic transit was summarized as the 
colic geometric center (GC) at specified times: the GC is the 
weighted average of counts in the different colonic regions [ascending 
(AC), transverse (TC), descending (DC), rectosigmoid (RS)] and 
stoof, respectively 1 to 5. Thus, at any time, the proportion of counts 
in each colonic region is multiplied by its weighting factor as follows:

\[
\begin{align*}
\%AC & = 1 + %TC \times 2 + %DC \times 3 + %RS \times 4 + %stoof \times 5 \\
& = 100 = \text{geometric center.}
\end{align*}
\]

Thus a higher GC reflects a faster colonic transit.

The primary transit end points were the gastric emptying t1/2 and 
colonic geometric center at 24 h (GC24). In 98 participants, only 
gastric emptying was performed with the same method and scans 
obtained over the first 4 h.

Gastric volume by 99mTc-SPECT. Gastric volume was measured in 
210 participants using a 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). 
This method uses SPECT (4, 25) after intravenous administration of 
99mTc-sodium pertechnetate (0.12 mCi/kg), which is taken up by the 
gastric mucosa (29). The camera (SMV-GF, Fairfax, CT) rotates 
around the thorax and abdomen with the participant lying in the 
supine position. The stomach was identified in the transaxial SPECT 
images and separated from background by a semiautomated segmen-
tation algorithm. A three-dimensional rendering of the stomach and its 
volume was obtained by using the AVW 3.0 (Biomedical Imaging 
Resource, Mayo Foundation, Rochester, MN) image-processing li-
braries. The primary end points were fasting and postprandial gastric 
volume.

Rectal sensation by barostat. These studies were conducted in 123 
subjects (91 patients with IBS and 32 healthy controls) who presented 
to the research center 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 (13). A catheter with a polyethylene bag (MUI Scientific, 
Mississauga, Ontario, Canada), was inserted into the rectum so that 
the middle of the balloon was ~10 cm from the anal verge. Subjects 
were placed in a semiprone position and the foot end of the bed was 
elevated 15°. The bag was then unfolded by transiently inflating it 
with 75 ml of air. The catheter was connected to a barostat (G&J 
Electronics, Toronto, Ontario, Canada) and the pressure in the bag 
was increased from 4 mmHg in steps of 1 mmHg for 1 min per step 
until respiratory excursions were observed. 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. This renders subsequent assessments of 
compliance and perception more reproducible (20). The bag was then 
deflated to 0 mmHg and the subjects were allowed to rest for 10 min. 
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
distention 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 were measured by using 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. R. 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. Subjects were asked to mark separate 100-mm visual analog scales (VAS) 30 s after the onset of the distension for the sensations of gas, urgency, and pain. These scales were anchored at the ends by the descriptions “unnoticeable” and “unbearable.” Pressure was released if the subject reported greater than 80 mm of pain on the VAS, and higher distensions were not subsequently administered. Immediately prior to the sensory testing the participants filled in 100 mm VAS assessing their current state of tiredness, worry, peace, and activity.

The following measurements were derived: 1) the sensory thresholds for first sensation, gas, urgency, and pain during ascending method of limits and 2) the gas, urgency, and pain scores in response to the four random phasic distensions (12, 24, 30, and 36 mmHg above baseline operating pressure) delivered in randomized order according to a scheme generated by the study statistician and communicated to the technologist on the day of the sensation test.

**FAAH genotyping.** FAAH genotype was determined by an investigator (P. Carlson) blinded to all clinical information using TaqMan SNP genotyping assays (Applied Biosystems) and confirmed by direct sequencing of representative samples. We adapted the approach previously published by Sipe et al. (33) in which primers were designed for FAAH C468W wild-type C and mutant A alleles.

**Statistical analysis.** The statistical analysis assessed the overall association of genotype with symptom phenotype by using contingency table analyses (\(x^2\) test), combining all FGIDs into one group and separately using the individual FGID subtypes. Odds ratios for each symptom phenotype (compared to controls) in FAAH CA/AA (relative to CC) were estimated by multiple logistic regression, adjusting for age and gender. Association of genotype with gastrointestinal motor function was assessed by analysis of covariance (ANCOVA), adjusting for age, gender, body mass index (BMI), and symptom phenotype (including healthy controls). The analysis of rectal sensation thresholds used proportional hazards regression models separately for each threshold type (first sensation, gas, urgency, and pain) incorporating hospital anxiety and depression (HAD) scores for anxiety and depression (40), BMI, and somatic symptom checklist score as covariates. The analysis of the sensation VAS ratings scores used repeated measures ANCOVA models (multiple pressure distension levels as the repeated factor) separately for gas, urgency, and pain sensation types and included the HAD scores, somatic symptom checklist score, BMI, symptom checklist-90 (SCL-90) somatization scale (15), and the scores for “tired,” “worried,” “peace,” and “activity” during the sensation test as covariates. Along with the main effects terms for phenotype and genotype, these models also included a genotype-by-phenotype interaction term. Post hoc pairwise comparisons of genotype subgroups within each phenotype were also examined.

**RESULTS**

**Demographics and distribution of FAAH genotype.** Participant demographics are included in Table 1. Genotype distribution did not deviate significantly from Hardy-Weinberg equilibrium at the polymorphic locus. The distribution of CC/CA/AA genotype in the healthy controls was similar to that reported in other predominantly Caucasian controls (18, 23; Fig. 1), that is, −60:30:5.

**Association of genotype with symptom phenotype.** There was a significant association of genotype with overall FGID phenotype compared with healthy controls (\(P = 0.011, x^2\)). There was also a significant association between FAAH genotype and individual phenotype (\(P = 0.048, \text{Fig. 2}\)); thus the odds for FGID subphenotype (compared with health) were increased in FAAH CA/AA (relative to CC) in D-IBS (\(P = 0.008\)) and M-IBS (\(P = 0.012\)). The association with chronic abdominal

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Table 1. **Participant demographics**

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>N</th>
<th>Sex (Female:Male)</th>
<th>Mean ± SD</th>
</tr>
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<tbody>
<tr>
<td>C-IBS</td>
<td>159</td>
<td>152:7</td>
<td>45.08 ± 14.39</td>
</tr>
<tr>
<td>D-IBS</td>
<td>184</td>
<td>153:31</td>
<td>45.16 ± 15.24</td>
</tr>
<tr>
<td>M-IBS</td>
<td>86</td>
<td>79:7</td>
<td>41.01 ± 13.31</td>
</tr>
<tr>
<td>Chronic abdominal pain</td>
<td>20</td>
<td>14:6</td>
<td>46.55 ± 15.77</td>
</tr>
<tr>
<td>Functional dyspepsia</td>
<td>33</td>
<td>13:20</td>
<td>52.54 ± 11.53</td>
</tr>
<tr>
<td>All FGID</td>
<td>482</td>
<td>411:71</td>
<td>44.95 ± 14.60</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>252</td>
<td>185:67</td>
<td>36.62 ± 12.43</td>
</tr>
<tr>
<td>FAAH CC</td>
<td>464</td>
<td>378:86</td>
<td>42.40 ± 14.41</td>
</tr>
<tr>
<td>FAAH CA/AA</td>
<td>270</td>
<td>218:52</td>
<td>41.57 ± 14.51</td>
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</table>

Note that, for the genotype subgroups, data are pooled for functional gastrointestinal disorder (FGID) patients and healthy volunteers. IBS, irritable bowel syndrome; C-IBS, functional constipation; D-IBS, functional diarrhea; M-IBS, mixed bowel function.
pain was not statistically significant ($P = 0.055$). Odds ratios from the logistic regression model were adjusted for age and gender.

**Association of genotype with motility phenotype.** There was no significant overall association of FAAH genotype with gastrointestinal motor function (Table 2, analysis of covariance adjusting for age, gender, and BMI).

However, there was a significant association with colonic transit (GC at 24 h) in D-IBS ($P = 0.037$), that is, FAAH CA/AA genotype was associated with accelerated colonic transit compared with FAAH CC genotype in patients with D-IBS. There was no significant association of FAAH genotype and gastric emptying (Fig. 3); on the other hand, the gastric accommodation volume (Fig. 4) appears to be possibly influenced by the FAAH genotype in dyspepsia ($P = 0.057$).

**Association of genotype with sensation phenotype.** There was no significant overall association of FAAH genotype with rectal sensation thresholds or rectal sensory ratings (Table 3) in response to random order distensions. No interaction effects were detected, indicating lack of associations between FAAH genotype and sensory ratings within different IBS phenotypes and healthy controls.

**DISCUSSION**

Our single-center study presents a number of novel perspectives. The study explored the association between genetic variation in endocannabinoid metabolism that controls, in part, CB1 receptor modulation and the occurrence of functional gastrointestinal disorders and the associated changes on motor or sensory functions. The significant associations of FAAH genotype with M-IBS and D-IBS symptoms and with colonic transit in D-IBS are consistent with the knowledge that CB1 receptor modulation alters colonic smooth muscle contraction and alters colonic tone and phasic contractions in vivo in humans (16, 21). These results support the hypothesis that genetic metabolism of endocannabinoids is associated with symptom phenotype in IBS and colonic transit in D-IBS. Our study in humans, in principle, is comparable to the use of knockout animals to demonstrate the importance of a certain mechanism through the observation of a certain function or phenotype. Knockout or silencing approaches are possible in experimental animals, but not in humans. Thus we use the natural variation in the genes controlling FAAH enzyme in humans to assess whether this genetic variation impacts on the phenotype of interest, and infer that cannabinoid mechanisms might be involved in the generation of that phenotype.

The association between genotype variation (non-wild-type CA or AA FAAH genotypes) in endocannabinoid metabolism by FAAH and colonic transit at 24 h in patients with D-IBS is concordant with the association with D-IBS or M-IBS symptom phenotype. This concordance supports the general hypothesis that endocannabinoids may be relevant to the control of mechanisms that result in symptoms of IBS and alteration in colonic motor function. The observation of accelerated colonic transit and phenotypes that include diarrhea suggest that, if the non-wild-type (CA/AA) genotype results in reduced expression of FAAH, the increased anandamide may ultimately block the release of transmitter from prejunctional inhibitory neurons (reviewed in Ref. 38). Similarly, it has been demonstrated that the CB1 agonist, WIN55212-2, causes a presynaptic depres-
sion of GABAergic inhibitory postsynaptic currents in hippocampal slices (19, 22), and reduces GABA release from the presynaptic boutons of local interneurons in the rostral ventromedial medulla (36).

Cannabinoid mechanisms may be relevant to several of the putative mechanisms of FGID. For example, IBS is associated with visceral hypersensitivity (6), and animal models of acute or chronic pain support a role of cannabinoid mechanisms in control of nociception in pain models, including that associated with gut inflammation (26, 30, 32). CB1 immunoreactivity is located on normal colonic epithelium, smooth muscle, and the myenteric plexus, whereas CB1 and CB2 receptors are expressed in plasma cells (39). IBS is associated with colonic motor dysfunction (6). In mice, endocannabinoids acting on myenteric CB1 receptors tonically inhibit colonic propulsion (31), and activation of CB1 receptors coupled to cholinergic motor neurons inhibits excitatory nerve transmission in human colonic circular muscle in vitro (21). A nonspecific CB receptor agonist, dronabinol, inhibits human gastric and colonic motor function in vivo (16, 17).

The cannabinoid system also modulates immune function and inflammation (32), and this may be relevant in a subset of patients with functional dyspepsia.

Table 3. Relationship between FAAH genotype and sensory thresholds for rectal sensation and rectal sensory ratings of gas, urgency, and pain in response to rectal distensions

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>FAAH Genotype</th>
<th>First Sensation</th>
<th>Gas</th>
<th>Urgency</th>
<th>Pain</th>
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<tr>
<td>Rectal sensation thresholds</td>
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<tr>
<td>C-IBS</td>
<td>25</td>
<td>CC</td>
<td>8</td>
<td>16</td>
<td>20</td>
<td>36</td>
</tr>
<tr>
<td>C-IBS</td>
<td>13</td>
<td>CA/AA</td>
<td>8</td>
<td>12</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>D-IBS</td>
<td>15</td>
<td>CC</td>
<td>8</td>
<td>12</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>D-IBS</td>
<td>12</td>
<td>CA/AA</td>
<td>6</td>
<td>10</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>M-IBS</td>
<td>12</td>
<td>CC</td>
<td>8</td>
<td>12</td>
<td>16</td>
<td>24</td>
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<tr>
<td>M-IBS</td>
<td>13</td>
<td>CA/AA</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>24</td>
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<tr>
<td>Health</td>
<td>21</td>
<td>CC</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>Health</td>
<td>11</td>
<td>CA/AA</td>
<td>8</td>
<td>8</td>
<td>16</td>
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<table>
<thead>
<tr>
<th>Rectal sensory ratings</th>
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<tbody>
<tr>
<td>Pressure, mmHg:</td>
<td>12</td>
<td>24</td>
<td>30</td>
<td>36</td>
<td>12</td>
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<tr>
<td>Gas</td>
<td></td>
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<td>Urgency</td>
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<tr>
<td>Pain</td>
<td></td>
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<tr>
<td>CC FAAH, n = 73</td>
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<tr>
<td>40.0±2.7</td>
<td>54.3±3.4</td>
<td>59.0±3.6</td>
<td>60.1±4.0</td>
<td>42.8±2.6</td>
<td>64.1±2.8</td>
</tr>
<tr>
<td>CA/AA FAAH, n = 50</td>
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<td>41.1±3.1</td>
<td>54.3±3.4</td>
<td>59.0±3.6</td>
<td>60.1±4.0</td>
<td>47.4±2.9</td>
<td>65.3±2.6</td>
</tr>
</tbody>
</table>

*Top: median threshold values in mmHg. Bottom: sensory ratings (means ± SE) in response to standard pressure distensions using random order phasic distensions (mmHg above baseline operational pressure). Note that there are no significant differences in either median thresholds or sensory ratings in healthy participants or patients with IBS who underwent rectal sensation studies (total n = 123).
patients with FGIDs (6). CB1 and CB2 receptor activation occurs in an experimental model of colitis induced by oil of mustard (25), and anti-inflammatory effects of cannabinoids may involve cytokines, chemokines, modulation of adenosine signaling (9), expression of adhesion molecules, and the migration, proliferation, and apoptosis of inflammatory cells (26, 37). Given these findings, future studies should also explore the potential role of cannabinoid mechanisms in the inflammatory component of IBS.

Strengths. Although the present study used a large database (almost 750) of well-characterized patients and healthy volunteers in a single center, as well as validated questionnaires and measurements of gastric and colonic functions, the results should be viewed as only hypothesis generating, given the retrospective analysis of the healthy volunteers and FGID patients who had participated in other studies that evaluated gastric and colonic motor and sensory functions, as well as the relatively small sample sizes of some of the patient groups. Thus it is possible that type II errors may contribute to the nonsignificant associations between FFAH variation and gastric volume in dyspepsia (n = 23, P = 0.06), and the odds ratio for an association between FFAH variation (as shown in Fig. 4) and CAP (n = 20, P = 0.055, as shown in Fig. 2).

The present data provide information that is useful to plan future studies to test the general hypothesis that FFAH presents a functionally relevant genetic variation in the control of gastrointestinal motor and sensory functions. Given the results in the present study, it would seem reasonable to focus on D-IBS and colonic transit and, possibly, on gastric accommodation in functional dyspepsia, especially the postprandial distress syndrome subgroup. There is biological evidence of functional relevance of this same genetic variation in the association with drug abuse and with overweight and obesity in Caucasians and African Americans (33, 34).

Limitations. Despite the fact that this sample is larger than any other prior study of genetic association with symptom phenotype in the IBS literature and is the first seeking an association between genotype and physiological phenotypes, a limitation of our study is that the prevalence of the homoygous A (AA) genotype is too small (5%) to allow an independent assessment of the association of AA genotype with phenotypes or motor functions with the sample size available. Therefore, a follow-up study is needed to determine whether AA genotype alters colonic transit relative to wild-type (CC) genotype. Another limitation is that it is unclear whether the heterozygous (CA) genotype reduces FFAH protein expression or function.

The only genetic modulation in the cannabinoid system assessed in this study pertains to the metabolism of the endocannabinoid, anandamide. The rationale for this choice was based on the prior literature showing functional significance of the genetic variant of FFAH (11) and the fact that, among the 13 SNPs in FFAH gene, the only one with a functional effect is theC385A (rs324420). By way of contrast, National Center for Biotechnology Information (NCBI) records >120 SNPs in the MGLL gene, which encodes for the rate-limiting enzyme monoacyl glycerol lipase for metabolism of 2-arachidonoyl glycerol, another endocannabinoid. Of these >120 SNPs, two are nonsynonymous SNPs, whereas the rest are synonymous or intronic up- or downstream from the gene. The two nonsynonymous genes are designated rs11538700 and rs1804711. In NCBI databases, there are no data on allelic distribution for rs1804711; for rs11538700, 58 European Caucasians, 44 Asians, and 58 sub-Saharan Africans showed no allelic changes, that is, all participants showed the TT genotype. Similarly, in reviewing the genetic variation in CNR1 (the gene for CB1 receptor), there are only two synonymous SNPs, rs35057475 and rs1049353. In NCBI, allelic frequency of rs35057475 has been reported for only 24 European Caucasians (A/G = 0.026, G/G = 0.974), and rs1049353 has been reported in 38 African Americans (A/G = 0.458; G/G = 0.542). For rs35057475, there were no homozygotes of the minor allele; for rs1049353, the minor allele frequency is 5%. There is an (AAT) repeat at the 3’ end of CNR1, 18,000 bases downstream from the start site of exon 4 of CNR1. The literature shows that the number of repeats is highly variable and, although associations with psychiatric disease, intravenous drug use, and response to antipsychotics have been described (2, 3, 12, 28, 35), the functional significance of the genetic variation is unclear.

In summary, FFAH genotype variation in the metabolism of endocannabinoids is associated with symptom phenotype in IBS and colonic transit in D-IBS. The effects of this genetic variation on gut function and responses to cannabinoid modulation deserve further study, including studies of familial associations of the genotype with symptoms suggestive of FGID in the apparently healthy relatives of IBS patients. In this study, we observed no statistically significant association between FFAH genotype and sensation measurements in IBS or health. However, further investigation of these mechanisms will be required using exogenous agents modulating CB receptor on models of visceral pain in patients with IBS to characterize more fully the potential therapeutic effects of cannabinoid agents.

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REFERENCES


