Candidate genes and sensory functions in health and irritable bowel syndrome

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Adrenergic and serotonergic (ADR-SER) mechanisms alter gut (GI) function; these effects are mediated through G protein transduction. Candidate genetic variations in ADR-SER were significantly associated with somatic scores in irritable bowel syndrome (IBS) and gastric emptying but not small bowel or colonic transit. Our aim was to assess whether candidate ADR-SER genes are associated with motor and sensory GI functions in IBS and subgroups on the basis of bowel dysfunction. In 122 patients with IBS and 39 healthy controls, we assessed gastrointestinal somatic symptoms and affect by validated questionnaires. We measured: gastric volume (GV), maximum tolerated volume, rectal compliance, sensation thresholds and ratings, and genetic variations in intestinal propulsion, coordinated motor activity in the small bowel or colon, and gastrointestinal tone and fluid secretion in intestinal transit in mice (40) and colonic circular smooth muscle in canine colon (47). The GLP-1 receptor and 5-HT3 receptor are differentially expressed in IBS and are associated with visceral pain perception (33). The GLP-1 receptor is involved in pain perception in the spinal cord and brain peripherally and to activate the hypothalamo-pituitary-adrenal axis (32). In humans, a combination of serotonin and norepinephrine reuptake inhibition has a more profound effect on human gastric and colonic sensory and motor functions relative to selective serotonin reuptake (14, 15). These and other data suggest that adrenergic and serotonergic mechanisms may interact to alter gastrointestinal motor and sensory functions, and these may involve G protein transduction.

Genetic variations in the mechanisms that control adrenergic receptors, G proteins, and the reuptake of serotonin are reported to be associated with different irritable bowel syndrome (IBS) or dyspepsia phenotypes (24, 28), associated somatic scores (28), and depressive episodes (25) although results are inconsistent (1, 44). In a previous study of symptom phenotype, we observed that single nucleotide polymorphisms (SNPs) in genes for α2C receptor and 5-HTTLPR (5-hydroxytryptamine transporter long polymorphic region in the promoter for serotonin transporter protein, SERT) either alone or in combination are associated with constipation-predominant IBS [IBS-C (28)] and with high somatic symptom scores in these patients (28). SERT is also termed SLC6A4. There is also evidence that the adrenergic and serotonergic systems interact in mediating functional dyspepsia (34).

In a recently published study of 251 patients with dyspepsia or IBS, or in healthy states, associations were demonstrated between candidate genes affecting adrenergic and serotonergic

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Adrenergic mechanisms alter gastrointestinal reflexes involved in intestinal propulsion, coordinated motor activity in the small bowel and colon, and gastrointestinal tone and fluid secretion (12, 46). Norepinephrine is also involved in control of pain including supraspinial inhibition of pain, e.g., due to a change in the behavioral state (37) including stress, which contributes to gut motility disorders (10).

Among α2 receptors, the α2A subtype appears to be critically important for control of motor functions, such as gastrointestinal transit in mice (40) and colonic circular smooth muscle in canine colon (47). The α2 receptor agonist, clonidine, inhibits gastric and colonic tone, phasic contractility, and sensation in the rectum and colon in response to balloon distension in healthy humans (4, 43).

Serotonergic (5-HT) mechanisms also alter gut absorptive, motor, and sensory functions via actions in the gut, spinal cord, and brain stem (27). Ligand-receptor interaction results in cellular functions with an estimated 80% of these interactions mediated by G proteins. Thus it is conceivable that genetic variation in G proteins may influence the effects of neurotransmitters including norepinephrine and serotonin. Holtmann et al. (22) observed an association between GNB3 genotype and dyspepsia though the association with a variety of functional gastrointestinal disorders was not confirmed (1).

It has been demonstrated that α2 adreceptors modulate neuronal serotonin release in mouse brain, suggesting that the two control mechanisms interact in the nervous system (39). Serotonergic and noradrenergic systems interact to modulate pain perception in the spinal cord and brain peripherally and to activate the hypothalamo-pituitary-adrenal axis (32). In humans, a combination of serotonin and norepinephrine reuptake inhibition has a more profound effect on human gastric and colonic sensory and motor functions relative to selective serotonin reuptake (14, 15). These and other data suggest that serotonergic and adrenergic mechanisms may interact to alter gastrointestinal motor and sensory functions, and these may involve G protein transduction.

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functions and gastric emptying or accommodation but not with small bowel or colonic transit (22).

Our hypothesis is that sensory functions in health and IBS are modulated by genetic variations in α2AR, 5-HTTLPR, and GNB3. Our aim was to assess whether genetic variations in adrenergic and serotonergic control and G protein are associated with sensory functions in health and IBS.

MATERIALS AND METHODS

Study Design

All participants underwent studies of satiation and rectal sensation, and, to facilitate interpretation of these sensory responses, we also measured gastric volumes and rectal compliance.

Participants and Questionnaires

This study recruited 163 participants, of which 161 provided DNA for studies. Thus a total of 122 patients with IBS (Rome II positive, 3 male) and 39 healthy controls were assessed. The study was approved by Mayo Clinic Institutional Review Board. All participants signed informed consent. To characterize the subtype of IBS on the basis of predominant bowel function, we used validated bowel symptom questionnaires (41), reviewed the electronic medical record (by S. McKinzie), or conducted direct physician (M. Camilleri) interview and examination. Participants were allowed to be on stable doses of the following medications: thyroid replacement, estrogen replacement, low-dose aspirin (81 mg/day), and birth control pills or depot estrogen injections. Exclusion criteria included use of medication for IBS or constipation within 7 days before measurements, any structural or metabolic diseases/conditions that affect the gastrointestinal system, or participation in another clinical study within the prior 30 days.

All participants underwent bowel disease questionnaires [including somatic symptom questionnaires (41)], hospital anxiety and depression inventory (HAD) (48), and a general quality of life instrument [SCL-90 (19)]. Participants also filled in questionnaires to assess their state of anxiety, relaxation, and fear of pain with the use of the 30-item Fear of Pain Questionnaire [FPQ-III (35)] and a revised version of the 36-item Anxiety Sensitivity Index [ASI-R (42)] on the days of rectal sensation tests. The observations using all of the same measurements, as well as full gastrointestinal transit, have been reported elsewhere (9). The results are only used here to assess association with candidate genes.

Satiation by the Nutrient Drink Test

A standardized nutrient drink test to measure satiation and postprandial symptoms was used (13). The method has been used extensively in previous studies, and a liquid nutrient drink, Ensure (1 Kcal/ml, 11% fat, 73% carbohydrate, and 16% protein), was used to identify maximum tolerated volume at full satiation, as well as postprandial fullness, nausea, bloating, and pain 30 min after the meal.

Gastric Volume by 99mTc-SPECT

We used a single photon emission computed tomography (SPECT) method (5) developed, validated, and extensively used in our laboratory to measure the gastric volume during fasting and after 300-ml liquid nutrients (300 kcal). The primary endpoint was postprandial change in gastric volume, and secondary endpoints were fasting and postprandial gastric volumes.

Rectal Compliance and Sensation by Barostat

Rectal barostat equipment and procedure. The method and performance characteristics have been extensively described elsewhere (17, 40). Ascending method of limits was used to measure rectal compliance and sensory thresholds. Random-order phasic distensions were used to assess sensory ratings, as in prior studies (4, 45). The methods are detailed in Fig. 1.

Methods for measuring rectal compliance and sensation. All subjects 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. A catheter, which was attached a polyethylene bag, was inserted into the rectum so that the middle of the balloon was located ~10 cm from the anal verge. To decrease the effects of abdominal viscera on the balloon volume, the subjects were placed in a semiprone position and the foot end of the bed elevated 15 degrees. The bag was unfolded by transient inflation with 75 ml of air, followed by complete deflation. After a 20–30-min recovery period, the catheter was connected to a barostat (G&J Electronics, Toronto, Ontario, Canada), and the pressure in the bag increased from 4 mmHg in steps of 1 mmHg for 1 min per step until respiratory excursions were observed. The baseline operating pressure (BOP) was set 2 mmHg above the minimal distension pressure at which respiratory excursions were clearly recorded from the barostat tracing. If respiratory variations were not seen by 18 mmHg, BOP was set at 12 mmHg. An initial “conditioning” distension of the rectum was then performed in which the pressure was increased from 0 mmHg in steps of 4 mmHg for 15 s per step until 20 mmHg was reached. Previous studies have shown that an initial conditioning distension to 20 mmHg renders subsequent assessments of compliance and perception more reproducible (23). The bag was then deflated to 0 mmHg, and the subjects were allowed to rest for 10 min before proceeding to the ascending method of limits. The experimental design is shown in Fig. 1.

Ascending method of limits: compliance and sensory thresholds. Rectal compliance and sensory thresholds were measured by ramp inflation, starting at 0 mmHg and increasing the pressure each minute in steps of 4 mmHg to a maximum of 60 mmHg. Thresholds for first sensation, gas, urgency, and pain (Fig. 1) were indicated by the subjects by pressing a button at the distension pressure at which sensations were perceived. Ramp inflation was terminated as soon as the subjects reported the first sensation of pain. Following this procedure, the bag was deflated to BOP and participants rested for 10 min.

Random-order phasic distensions: sensory ratings. After the protocol of ascending method of limits, phasic distensions of 12, 24, 30, and 36 mmHg above BOP were each applied once in random order. Each distension was maintained for 1 min with an interstimulus interval of 2 min during which the balloon was deflated to BOP. This approach has been shown to be reliable in multiple previous studies (4, 14, 15, 45) because the intensity ratings are generally proportional to the magnitude of the distension pressures. Study participants were blinded to the distension order, which was provided by the study.
statistician (A. R. Zinsmeister). Participants 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 each end by the descriptions “unnoticeable” and “unbearable.” Pressure was immediately released if the subject reported greater than 80 mm of pain on the VAS scale, and higher distension pressures were not subsequently administered. During the assessment of sensation, the interaction between the subject and the study investigator (I. Busciglio) was kept to a minimum.

Data analysis. The following measurements were obtained: 1) the sensory thresholds for first sensation, gas, urgency, and pain during ascending method of limits, 2) the three individual sensation scores (gas, urgency, and pain) in response to the three random phasic distensions (13, 25, 32, and 36 mmHg above BOP), and 3) rectal compliance.

Rectal pressure-volume relationships were analyzed with the use of a linear interpolation method that was recently described and validated (21). A summary value for each compliance curve was thus calculated for each subject, specifically the pressure observed at one-half of the maximum observed volume (Pr1/2), where a smaller Pr1/2 corresponds to higher compliance.

Candidate Genes Tested via SNPs

SNPs tested were: a2A (C-1291G), a2C (Del 322–325), 5-HTTLPR, and GNβ3 (C825T). The methods have been published in prior reports from this laboratory (1, 22, 28).

Data and Statistical Analysis

The initial analysis examined the overall association of symptom phenotype (health vs. overall IBS), and separately the four phenotypes [health, IBS-C, diarrhea-predominant IBS (IBS-D), and mixed bowel dysfunction IBS] with each genotype using contingency table analyses (chi-square test or Fishers exact test as warranted). The primary endpoints for evaluating the association of genetic variations with sensory function in IBS and health were maximum tolerated nutrient drink volume (MTV) and symptoms, sensory thresholds, and ratings in response to distensions. The secondary endpoints were fasting and postprandial gastric volumes [as surrogates for the tone of the stomach]. Sensory thresholds for first sensation, gas, urgency, and pain during response to distensions. The secondary endpoints were fasting and postprandial gastric volumes [as surrogates for the tone of the stomach], and rectal compliance.

Analyses were done separately for each candidate gene. In the analyses of the individual physiologic response endpoints, the genotypes were categorized as wild-type vs. nonwild-type and were included as predictor variables of these sensory functions in separate analyses of covariance (ANCOVA) or proportional hazards regression models (for the sensory thresholds to account for the small number of “censored” values, i.e., the maximum distension did not evoke the particular sensory type). In addition, a genotype by disease group (IBS vs. health) interaction term was included in each of the models to examine whether the association of genotype with sensory function was similar in patients with IBS vs. healthy subjects. A repeated measures ANCOVA (for the repeated distension levels) was used to assess the association of genotype with sensory VAS ratings separately for sensations of gas, urgency, and pain. The following covariates were included in the repeated measures models: age, body mass index (BMI), HAD scale scores, SCL-90-R somatization scale score, the somatic symptom checklist (SSC) score, and, for the sensory threshold models, the ratings for “tired,” “worried,” “peace,” and “active” attributes.

The aim in these hypothesis-generating analyses was to explore potential associations that would warrant further study, and thus no adjustment in the 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, and thus no adjustment in the 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, and thus no adjustment in the 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, and thus no adjustment in the 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, and thus no adjustment in the alpha level for multiple tests was made.

Some genotypes such as 5-HTTLPR LS and SS were grouped because of the low prevalence (<20%) of SS genotype in people in the Midwest of the United States who are predominantly of Northern European extraction (28). With the sample size available, the study was not adequately powered to detect potential associations with SS genotype alone. Moreover, the prior observation by Lesch et al. (29) suggested that the S allele is associated with decreased production of the serotonin transporter protein, justifying the combination LS/SS. Similarly, the prevalence of GG a2A is 7% and TT GNβ3 is 3% in controls studied from the same geographical region and similar ethnicity in the Midwest USA around southeastern Minnesota (1, 28).

RESULTS

Participants. Table 1 shows key demographics and psychological data in 161 participants (39 controls and 122 patients

| Table 1. | Key demographics, psychological and genotype data in 161 participants |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Number (no. male) | Healthy Controls | C-IBS | D-IBS | M-IBS |
| Age, yr | 39.0 | 38.4±1.5 | 35.4±1.6 | 37.2±2.2 |
| BMI, kg/m² | 25.1±0.7 | 25.1±0.4 | 28.9±1.0 | 27.9±0.8 |
| Anxiety score, HADS | 3.2±0.4 | 4.7±0.5 | 5.0±0.5 | 5.0±0.4 |
| Depression score, HADS | 0.6±0.2 | 1.6±0.3 | 1.4±0.2 | 1.9±0.4 |
| Somatization T score | 41.3±1.0 | 47.6±0.9 | 49.3±1.2 | 48.8±1.5 |
| SCL-90 general severity index T score | 37.6±1.3 | 44.0±1.3 | 44.6±1.5 | 44.6±1.7 |
| Somatic symptom score | 0.3±0.05 | 0.4±0.04 | 0.5±0.05 | 0.4±0.05 |
| Number taking SSRI, stable dose | 5 | 9 | 11 | 6 |
| Genotype | | | | |
| a2A CC, n (%) | 25 (64.1) | 21 (42.9) | 24 (54.6) | 12 (41.4) |
| a2A GC/GG, n (%) | 14 (35.9) | 28 (57.1) | 20 (45.4) | 17 (58.6) |
| a2C* wild-type, n (%) | 38 (97.4) | 45 (91.8) | 42 (95.4) | 25 (86.2) |
| a2C* (Del 322–325), n (%) | 1 (2.6) | 4 (8.2) | 2 (4.6) | 2 (6.4) |
| GNβ3 CC, n (%) | 19 (48.7) | 27 (55.1) | 19 (43.2) | 11 (37.9) |
| GNβ3 TC/TT, n (%) | 20 (51.3) | 22 (44.9) | 25 (56.8) | 18 (62.1) |
| SCL/C6a4 LL, n (%) | 16 (41.0) | 21 (42.9) | 16 (36.4) | 7 (24.1) |
| SCL/C6a4 LS/SS, n (%) | 23 (59.0) | 28 (57.1) | 28 (63.4) | 22 (75.9) |

Applicable values are means ± SE. *Not considered in further analyses due to small numbers of a2C (Del 322–325). IBS, irritable bowel syndrome; C-IBS, constipation-predominant IBS; D-IBS, diarrhea-predominant IBS; M-IBS, mixed bowel dysfunction IBS; BMI, body mass index; HADS, hospital anxiety and depression scale; SCL-90, symptom check list 90 revised; SSRI, selective serotonin reuptake inhibitor.
with IBS, the latter categorized by IBS subgroup on the basis of bowel function. Data on gastrointestinal transit, rectal sensation, and compliance for each of these groups have been published elsewhere (9).

Association between candidate genes and symptom phenotype. The prevalence of the α2C (Del 322–325) was <6%, providing insufficient power to explore associations of interest in this study.

There was a significant association between 5-HTTLPR genotype and subject phenotype (P = 0.034); specifically, higher proportions of subjects with the LL or the LS genotype were in the constipation phenotype; in contrast, a higher proportion of subjects with the 5-HTTLPR SS genotype were in the healthy group. A significant association between phenotype and the other two genotypes of interest, GNB3 and α2A, was not observed (for either overall IBS vs. health or the four phenotype subgroups). Although IBS status was moderately associated with SCL-90-R scores (partial $r^2 = 12\%$, $P < 0.001$) and modestly associated with somatic symptom, anxiety, and depression scores (partial $r^2$ values about 3–4%, $P < 0.05$), no association of genotypes with these scores was observed.

**Fasting gastric volumes.** GNB3 was associated with fasting gastric volume, with the TC/TT genotype being associated with lower fasting gastric volume (Fig. 2, $P = 0.03$); the association with postprandial change in gastric volume was not significant ($P = 0.095$).

**Satiation and postprandial gastric volumes.** There was no significant association between satiation volume, aggregate symptom scores, or postprandial changes in gastric volume and either of the three candidate genes of interest in participants with IBS and healthy participants.

**Rectal compliance, thresholds, and sensation ratings in different IBS groups and health.** In Table 2, rectal compliance, thresholds, and sensation ratings are summarized for healthy participants and IBS patients; these data are further tabulated for the combined group on the basis of specific genotype of interest (Table 3). Note that the only significant associations are for 5-HTTLPR genotype with rectal compliance (Fig. 3) and sensation ratings for pain (Table 3, Fig. 4), with LS/SS genotype being associated with lower Pr1/2 (signifying higher compliance) and higher pain ratings. Figure 4 shows that the association of pain ratings and 5-HTTLPR genotype is predominantly noted at 12 and 24 mmHg distensions.

**DISCUSSION**

This study has provided novel observations on the association of sensory functions in IBS and candidate genes of interest. Our study also explored the potential association of these candidate genes and symptom phenotype. We generally confirmed the prior studies from our laboratory (1, 28) regarding the lack of association of the three genotypes individually and IBS symptom phenotype. Thus a significant association between phenotype and the other two genotypes of interest, GNB3 and α2A, was not observed. However, there was a higher proportion of subjects with the 5-HTTLPR LL and LS genotype with the constipation phenotype, whereas a higher proportion of subjects with the SS genotype was in the healthy group. In general, the long allele was associated with more effective 5-HT reuptake (29), and it is conceivable that this reduces effects of endogenous 5-HT on motor and secretory functions that may ultimately lead to constipation.

The overall conclusions reached in the prior study from our laboratory (28) of the association of SERT genotype and IBS were confirmed by a recent systematic review and meta-analysis by van Kerkhoven et al. (44) that included patients of European or Asian extraction. Moreover, our present data showed that 5-HTTLPR SS genotype relative to combined LL/LS genotype was protective from association with IBS. This is also consistent with the findings in the studies by Pata et al. (36) and Niesler et al. (33), although the Peto plot in the paper by van Kerkhoven et al. (44) showed that an overall odds ratio for the association of SS genotype with IBS in the 8 studies in the literature was very close to 1.

Although IBS status was moderately associated with somatization scores (by SCL-90-R) and modestly associated with somatic symptoms and anxiety and depression scores (9), no association of genotypes with these scores was observed in the present study. Thus our present data do not appear to confirm that SLC6A4 SS genotype is more likely to be associated with depression among patients with IBS, as was previously reported by another group (25). However, it is important to note that the proportion of patients on antidepressants in our patient

<table>
<thead>
<tr>
<th>Endpoint†</th>
<th>Health, n = 39</th>
<th>IBS, n = 122</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compliance</td>
<td>Pr1/2, mmHg</td>
<td>(37) 12.3±0.8 (118) 13.5±0.4</td>
</tr>
<tr>
<td></td>
<td>Maximum balloon volume, ml</td>
<td>(37) 234±16 (118) 238±10</td>
</tr>
<tr>
<td>Sensation ratings</td>
<td>Gas at 30 mmHg</td>
<td>(36) 63±4 (111) 59±2</td>
</tr>
<tr>
<td></td>
<td>Gas at 36 mmHg</td>
<td>(33) 66±5 (106) 62±3</td>
</tr>
<tr>
<td></td>
<td>Pain at 30 mmHg</td>
<td>(36) 56±5 (111) 54±3</td>
</tr>
<tr>
<td></td>
<td>Pain at 36 mmHg</td>
<td>(33) 60±4 (106) 57±3</td>
</tr>
<tr>
<td></td>
<td>Urgency at 30 mmHg</td>
<td>(36) 74±3 (110) 73±2</td>
</tr>
<tr>
<td></td>
<td>Urgency at 36 mmHg</td>
<td>(33) 81±2 (105) 76±2</td>
</tr>
</tbody>
</table>

† Values are means ± SE; *means ± SE ignoring censored status. Pr1/2, pressure observed at one-half of the maximum observed volume. Number in parentheses refers to number of participants undergoing the study.
LS/SS genotype is associated with higher compliance (lower Pr1/2, primarily on the associations of these candidate genes with base of people who had undergone gastrointestinal motility dyspepsia (22). Those participants were selected from a data-mixed bowel habit, 49 IBS-C, 67 IBS-D, and 33 functional male and 191 female): 82 healthy, 20 patients with IBS with related to an association with abnormal gastric functions. Data show least square means adjusted for age, BMI, somatization, and group association between

In a previous study (22), we also observed significant genotype with dyspepsia in two reports in the literature (24, 28). The association of \( GN3 \) genotype and fasting gastric volume (with \( 5\text{-HTTLPR} \) genotype and fasting gastric fasted gastric volume (with \( 5\text{-HTTLPR} \) sensory endpoints. Significant genotype-function associations were noted for fasting gastric volume (with \( 5\text{-HTTLPR} \) specific candidate gene is demonstrated for a motor function, it is important to interpret with caution the association of that gene with a sensory function. In this study, we observed associations of increased rectal compliance (lower Pr1/2) and increased pain sensation ratings during rectal distension in those with \( 5\text{-HTTLPR} \) LS/SS genotype relative to LL genotype. A decreased compliance may contribute to higher pain

Table 3. Relationship of genotype to satiation volume, gastric volumes, rectal compliance, sensation ratings, and thresholds

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>( \alpha2A ) CC</th>
<th>( \alpha2A ) GC/GG</th>
<th>( GN3 ) CC</th>
<th>( GN3 ) TC/TT</th>
<th>SLC6A4 LL</th>
<th>SLC6A4 LS/SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>82</td>
<td>79</td>
<td>76</td>
<td>85</td>
<td>60</td>
<td>101</td>
</tr>
<tr>
<td>Satiation (MTV) volume, 1</td>
<td>1.03 ± 0.05</td>
<td>1.06 ± 0.03</td>
<td>1.02 ± 0.03</td>
<td>1.08 ± 0.04</td>
<td>1.07 ± 0.04</td>
<td>1.04 ± 0.03</td>
</tr>
<tr>
<td>Fasting gastric volume, ml</td>
<td>224 ± 7</td>
<td>240 ± 10</td>
<td>244 ± 10</td>
<td>221 ± 6</td>
<td>232 ± 11</td>
<td>232 ± 7</td>
</tr>
<tr>
<td>( \Delta ) Postmeal gastric vol, ml</td>
<td>521 ± 9</td>
<td>516 ± 10</td>
<td>507 ± 10</td>
<td>528 ± 9</td>
<td>513 ± 10</td>
<td>521 ± 9</td>
</tr>
<tr>
<td>Compliance, Pr1/2, mmHg</td>
<td>13.0 ± 0.5</td>
<td>13.4 ± 0.6</td>
<td>13.0 ± 0.6</td>
<td>13.3 ± 0.5</td>
<td>14.3 ± 0.6*</td>
<td>12.6 ± 0.5</td>
</tr>
<tr>
<td>Sensation rating gas at 30 mmHg</td>
<td>61 ± 3</td>
<td>59 ± 3</td>
<td>63 ± 3</td>
<td>58 ± 3</td>
<td>56 ± 3</td>
<td>63 ± 3</td>
</tr>
<tr>
<td>Rating gas at 36 mmHg</td>
<td>65 ± 4</td>
<td>61 ± 3</td>
<td>65 ± 4</td>
<td>61 ± 3</td>
<td>59 ± 4</td>
<td>65 ± 3</td>
</tr>
<tr>
<td>Rating pain at 30 mmHg</td>
<td>56 ± 3</td>
<td>53 ± 3</td>
<td>56 ± 4</td>
<td>53 ± 3</td>
<td>50 ± 4</td>
<td>57 ± 3</td>
</tr>
<tr>
<td>Rating pain at 36 mmHg</td>
<td>59 ± 4</td>
<td>56 ± 3</td>
<td>58 ± 4</td>
<td>58 ± 3</td>
<td>54 ± 4</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>Rating urgency at 30 mmHg</td>
<td>76 ± 2</td>
<td>71 ± 2</td>
<td>75 ± 2</td>
<td>76 ± 2</td>
<td>72 ± 2</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>Rating urgency at 36 mmHg</td>
<td>80 ± 2</td>
<td>74 ± 2</td>
<td>78 ± 2</td>
<td>74 ± 2</td>
<td>77 ± 2</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>Threshold† 1st sensation, mmHg</td>
<td>8.1 ± 0.5</td>
<td>8.0 ± 0.5</td>
<td>7.3 ± 0.4</td>
<td>8.7 ± 0.5</td>
<td>8.3 ± 0.6</td>
<td>7.8 ± 0.4</td>
</tr>
<tr>
<td>Threshold gas, mmHg</td>
<td>13.5 ± 0.8</td>
<td>14.1 ± 1.1</td>
<td>12.5 ± 0.8</td>
<td>14.9 ± 1.0</td>
<td>14.0 ± 1.1</td>
<td>13.7 ± 0.9</td>
</tr>
<tr>
<td>Threshold urgency, mmHg</td>
<td>18.0 ± 1.0</td>
<td>19.3 ± 1.0</td>
<td>17.6 ± 0.9</td>
<td>19.6 ± 1.0</td>
<td>19.2 ± 1.1</td>
<td>18.3 ± 0.9</td>
</tr>
<tr>
<td>Threshold pain, mmHg</td>
<td>29.6 ± 1.5</td>
<td>30.1 ± 1.4</td>
<td>29.1 ± 1.5</td>
<td>30.5 ± 1.4</td>
<td>32.0 ± 1.6</td>
<td>28.6 ± 1.3</td>
</tr>
</tbody>
</table>

Applicable values are means ± SE. *P < 0.05 vs. SLC6A4 LS/SS; †mean ± SE (includes censored values). MTV, maximum tolerated volume.

cohort was low, and we have previously demonstrated by a detailed analysis (9) that the patient cohort included in this study was less psychologically compromised than a series of patients with IBS studied at another referral center (20).

The association of \( GN3 \) genotype and fasting gastric volume is consistent with epidemiological association of \( GN3 \) genotype with dyspepsia in two reports in the literature (24, 28). In a previous study (22), we also observed significant association between \( GN3 \) genotype and gastric emptying of solids at 4 h. The physiological factors that are significantly associated with the development of postprandial symptoms in dyspepsia are fasting gastric volume and accelerated gastric emptying at 1 h and delayed gastric emptying at 4 h (18). Together with the previous study (22), our present observations on the association of \( GN3 \) genotype and fasting gastric volume are consistent with the hypothesis that the epidemiological association of \( GN3 \) genotype with dyspepsia may be related to an association with abnormal gastric functions.

We have previously observed associations of the same genetic variations with gastric emptying in 251 participants (60 male and 191 female): 82 healthy, 20 patients with IBS with mixed bowel habit, 49 IBS-C, 67 IBS-D, and 33 functional dyspepsia (22). Those participants were selected from a database of people who had undergone gastrointestinal motility tests. On the other hand, the present prospective study focused primarily on the associations of these candidate genes with sensory functions in health and in patients with IBS. In general, the satiation volume and postprandial gastric volumes were not significantly different in health and this group of patients with IBS (whole group and subtypes). As described and discussed extensively elsewhere (9), we found that ~16% demonstrated rectal hyposensitivity, ~21% demonstrated hypersensitivity, and the remainder demonstrated normosensitivity in this cohort of IBS patients relative to the concurrently recruited controls, all of whom were female. This study focused on relationships between the candidate genes and sensation in IBS, specifically satiation and rectal sensation. Associations with gastric volume and rectal compliance were of secondary interest and were included to facilitate interpretation of any associations identified between genotype and the sensory endpoints.

Significant genotype-function associations were noted for fasting gastric volume (with \( GN3 \), rectal compliance, and pain sensation (with \( 5\text{-HTTLPR} \)). When the association with a specific candidate gene is demonstrated for a motor function, it is important to interpret with caution the association of that gene with a sensory function. In this study, we observed associations of increased rectal compliance (lower Pr1/2) and increased pain sensation ratings during rectal distension in those with \( 5\text{-HTTLPR} \) LS/SS genotype relative to LL genotype. A decreased compliance may contribute to higher pain

Fig. 3. Rectal compliance in health and IBS on the basis of SLC6A4 gene. The LS/SS genotype is associated with higher compliance (lower Pr1/2, \( P = 0.051 \)). Data show least square means adjusted for age, BMI, somatization, and group (health vs. IBS).

Fig. 4. Association of SLC6A4 genotype with pain sensation ratings at the different levels of distension (\( P = 0.052 \) for overall test across all 4 distension levels); note that the LL genotype is associated with lower pain sensation ratings especially with 12 and 24 mmHg (above BOP) distensions.
sensation ratings (6). However, since we observed increased compliance and higher pain sensation ratings, we conclude that the influence of 5-HTTLPR LS/SS genotype on rectal compliance does not explain the increase in pain sensation observed with this genotype.

Therefore, this supports the hypothesis that 5-HTTLPR LS/SS genotype increases pain sensation. The association of the genotype with increased pain ratings is not observed with the higher distension pressures, probably reflecting a “ceiling” effect, that is, that 30 and 36 mmHg above BOP (see Fig. 4 and data in Table 3) constitutes a significant stimulus that over-whelms the hypothetical effect of the 5-HTTLPR genotype proposed here. The LS/SS genetic variation would be expected to reduce reuptake of serotonin, and, if the latter is mediating pain sensation, it would result in higher pain sensation ratings.

It is unclear whether the lack of a significant association of 5-HTTLPR LS/SS genotype with sensations of gas and urgency reflects a true biological difference in the influence of SLC6A4 on sensation. It is conceivable that these sensations are mediated by different neural pathways, but this requires further study to explore whether, apart from the genotype, the tissue expression of these mechanisms is also associated with motor and sensory functions in IBS. This concept has been explored in experimental animal studies.

In studies conducted in guinea pigs with 2, 4, 6-trinitrobenzenesulfonic acid-induced colitis, mucosal SERT (SLC6A4) expression was reduced, and this led to increased 5-HT availability and motor functions (30). The role of SERT expression in tissues from patients with IBS is still unclear. Although Bellini et al. (3) suggested that platelet SERT should reflect colonic SERT in IBS and observed a significant relationship with IBS-D, the assumption has not been validated or rigorously tested. Coates et al. (16) suggested that there was an association between IBS-C or IBS-D and reduced SERT mRNA mucosal expression; however, we were unable to replicate the association between IBS symptom phenotype and SERT mRNA in sigmoid colon mucosa (7).

The lack of association of α2A genotype on sensation of gas, urgency, or pain is not altogether surprising since upregulation of α2A receptors and norepinephrine appears to be mostly associated with injury (37), which may be less relevant in IBS. Injury induces expression of novel noradrenergic receptors, sprouting of sympathetic nerve fibers, and pronociceptive changes in the ionic channel properties of primary afferent nociceptors. α2C adrenoceptors on axon terminals of excitatory interneurons of the spinal dorsal horn possibly contribute to spinal control of pain (38); however, the genetic variation in the α2C receptor is too infrequent to allow us to explore its contribution to the endophenotype. On the other hand, α2A receptors appear to be more important in gastrointestinal motor functions (40, 47).

We perceive that studies of physiological genetics or “endophenotype” may help identify susceptibility genes for complex inherited traits like IBS, as has been reported in common mental disorders such as schizophrenia, bipolar disorder, and severe major depression. In all of these complex, multifactorial conditions, identification of disease-promoting genes may be facilitated by studies of disturbed functions rather than symptom phenotype. The schizophrenia literature illustrates the usefulness of endophenotypes in genetic analyses of mental disorders (11) and in understanding these disorders at the cellular and molecular levels (2). As with those mental disorders, there is evidence from familial clustering and twin studies to support a heritable component in IBS (26), although this is somewhat controversial (31). Although our laboratory focuses predominantly on peripheral organ functions, investigation of genetic influences on brain structure or functions in the development of IBS using neuroimaging may provide further advances in understanding, as in schizophrenia (2).

In conclusion, we propose that such studies, as well as a recent report on the effect of genetic control of cannabinoid metabolism on gut functions (8), may usher in studies of endophenotype in IBS and help decipher the role of genetics and candidate mechanisms in the etiology, pathophysiology, or manifestations of IBS.

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