Candidate Genes and Sensory Functions in Health and Irritable Bowel Syndrome

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Running head: Genes and sensorimotor function in IBS

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Abbreviations:

BOP = baseline operating pressure
Δ = delta (change e.g. in gastric volume postprandially)
GV: gastric volume
HAD: Hospital anxiety and depression inventory
5-HTTLPR: serotonin transporter linked polymorphic region
IBS: irritable bowel syndrome
IBS-C: constipation-predominant IBS
IBS-D: diarrhea-predominant IBS
IBS-M: mixed bowel dysfunction IBS
MTV: maximum tolerated volume, also termed satiation volume
SCL-90 (R): symptom check list 90 revised
SERT: serotonin transporter
SLC6A4: solute carrier family 6 (neurotransmitter transporter, serotonin), member 4
SNP: single nucleotide polymorphism
ABSTRACT (252 words)

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 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 based on bowel dysfunction. In 122 IBS patients and 39 healthy controls, we assessed gastrointestinal, somatic symptoms and affect by validated questionnaires. We measured: gastric volume (GV), maximum tolerated volume [MTV]); rectal compliance, sensation thresholds and ratings, and genetic variations: \( \alpha_2A \) (C-1291G), \( \alpha_2C \) (Del 332-325), \( GN\beta3 \) (C825T) and 5-HTTLPR. Demographics and genotype distributions were similar in the IBS patients subgrouped on bowel function. There were significant associations between 5-HTTLPR SS genotype and absence of IBS symptoms and between 5-HTTLPR LS/SS genotype and increased rectal compliance and increased pain ratings, particularly at 12 and 24 mmHg distensions. \( GN\beta3 \) was associated only with fasting GV; we did not detect associations between \( \alpha_2A \) genotype and the gastrointestinal sensory or motor functions tested. We concluded that 5-HTTLPR LS/SS genotype is associated with both increased pain sensation and increased rectal compliance though the latter effect is unlikely to contribute to increased pain sensation ratings with LS/SS genotype. The data suggest the hypotheses that the endophenotype of visceral hypersensitivity in IBS may be partly related to genetic factors and the association of \( GN\beta3 \) with fasting GV may explain, in part, the reported association of \( GN\beta3 \) with dyspepsia.

Key words: adrenergic, serotonergic, \( GN\beta3 \), SLC6A4, G protein, receptor
BACKGROUND

Adrenergic mechanisms alter gastrointestinal reflexes involved in intestinal propulsion, coordinated motor activity in the small bowel and colon, gastrointestinal tone and fluid secretion (12,46). Norepinephrine is also involved in control of pain including supraspinal 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 brainstem (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 observed an association between GNβ3 genotype and dyspepsia (22), though the association with a variety of functional gastrointestinal disorders was not confirmed (1).

It has been demonstrated that α2-adrenoceptors 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, 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.

Genetic variations in the mechanisms that control adrenergic receptors, G-proteins, and the reuptake of serotonin are reported to be associated with different 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 $\alpha_2C$ receptor and 5-HTTLPR (promoter for serotonin transporter protein, SERT, also named SLC6A4) either alone or in combination are associated with constipation-predominant IBS [IBS-C (28)] and with high somatic symptom scores in these patients (28). 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, IBS or health, associations were demonstrated between candidate genes affecting adrenergic and serotonergic 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 $\alpha_2AR$, 5-HTTLPR, and $\text{GN}\beta 3$. 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, in order 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 IBS patients (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 based on predominant bowel function, we used validated bowel symptom questionnaire (41), reviewed the electronic medical record (by SM), or conducted direct physician (MC) 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 prior to 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 (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 using 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, 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 minutes after the meal.

**Gastric Volume by $^{99m}$Tc-SPECT**

We used a SPECT method (5) developed, validated, and extensively used in our laboratory to measure the gastric volume during fasting and post-300 ml liquid nutrient (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 Figure 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 hour before reporting to the center) and an overnight fast. A catheter, to which was attached a polyethylene bag, was inserted into the rectum so that the
middle of the balloon was located approximately 10 cm from the anal verge. To decrease the effects of abdominal viscera on the balloon volume, the subjects were placed in a semi-prone 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 minute recovery period, the catheter was connected to a barostat (G&J Electronics Inc., Toronto, Ontario, Canada) and the pressure in the bag increased from 4 mmHg in steps of 1 mmHg for 1 minute 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 seconds 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 minutes before proceeding to the ascending method of limits. The experimental design is shown in Figure 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 (Figure 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 minutes.
Random Order Phasic Distensions: Sensory Ratings

After the ascending method of limits’ protocol, phasic distensions of 12, 24, 30 and 36 mmHg above BOP were each applied once in random order. Each distension was maintained for 1 minute with an inter-stimulus interval of 2 minutes during which the balloon was deflated to BOP. This approach has been shown to be reliable in multiple previous studies, as the intensity ratings are generally proportional to the magnitude of the distension pressures (4,14,15,45). Study participants were blinded to the distension order, which was provided by the study statistician (ARZ). Participants were asked to mark separate 100 mm visual analog scales (VAS) 30 seconds 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 (IB) was kept to a minimum.

Data Analysis

The following measurements were obtained: (i) the sensory thresholds for first sensation, gas, urgency, and pain during ascending method of limits, (ii) 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 (iii) rectal compliance.

Rectal pressure-volume relationships were analyzed using 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 ($P_{R_{1/2}}$), where a smaller $P_{R_{1/2}}$ corresponds to higher compliance.
Candidate Genes Tested via Single Nucleotide Polymorphisms (SNPs)

SNPs tested were: $\alpha_2A$ (C-1291G), $\alpha_2C$ (Del 332-325), 5HTTLPR, and $\text{GN}\beta\beta_3$ (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 versus overall IBS), and separately, the four phenotypes (health, C-IBS, D-IBS, and M-IBS), with each genotype using contingency table analyses (chi-square test or Fisher’s 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 which are factors that influence satiation in dyspepsia (18)] 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 wildtype vs. non-wildtype 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 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, BMI, HAD anxiety and
depression 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 which 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 with larger numbers of subjects and were specifically reported.

Some genotypes such as 5HTTLPR LS and SS were grouped because of the low prevalence (<20%) of SS genotype in people in the mid-West 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 the S allele is associated with decreased production of the serotonin transporter protein justifying the combination LS/SS. Similarly, the prevalence of GG α2A is 7% and TT GNβ3 is 3% in controls studied from the same geographical region and similar ethnicity in the mid-west USA around Southeastern MN (1, 28).

**RESULTS**

**Participants**

Table I shows key demographics and psychological data in 161 participants (39 controls and 122 patients with IBS, the latter categorized by IBS subgroup based on 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 $\alpha_{2C}$ Del 322-325 was <6%, providing insufficient power to explore associations of interest in this study.

There was a significant association between $5HTTLPR$ 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 $5HTTLPR$ SS genotype were in the healthy group. A significant association between phenotype and the other two genotypes of interest, $G\beta3$ and $\alpha_{2A}$, 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-squared=12%, $p<0.001$) and modestly associated with somatic symptom, anxiety and depression scores (partial r-squared values about 3-4%, $p<0.05$), no association of genotypes with these scores was observed.

Fasting Gastric Volumes

$G\beta3$ was associated with fasting gastric volume, with the TC/TT genotype being associated with lower fasting gastric volume (Figure 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 IBS and healthy participants.

Rectal Compliance, Thresholds and Sensation Ratings in Different IBS Groups and Health

In Table II, rectal compliance, thresholds and sensation ratings are summarized for healthy participants and IBS patients; these data are further tabulated for the combined group...
based on specific genotype of interest (Table III). Note that the only significant associations are for 5HTTLPR genotype with rectal compliance (Figure 3) and sensation ratings for pain (Table III, Figure 5), 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 5HTTLPR 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, \( \text{GN} \beta 3 \) and \( \alpha 2A \), was not observed. However, there was a higher proportion of subjects with the 5HTTLPR LL and LS genotype with the constipation phenotype, while 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. that included patients of European or Asian extraction (44). Moreover, our current data showed that 5HTTLPR 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, anxiety and depression scores (9), no association of genotypes with these scores was observed in the current study. Thus, our current 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 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 GNβ3 genotype and fasting gastric volume is consistent with epidemiological association of GNβ3 genotype with dyspepsia in two reports in the literature (24,28). In a previous study (22), we also observed significant association between GNβ3 genotype and gastric emptying of solids at 4 hours. 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 hour and delayed gastric emptying at 4 hours (18). Together with the previous study (22), our current observations on the association of GNβ3 genotype and fasting gastric volume is consistent with the hypothesis that the epidemiological association of GNβ3 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 IBS with mixed bowel
habit, 49 C-IBS, 67 D-IBS 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% hypersensitivity, and the remainder normosensitivity in this cohort of IBS patients relative to the concurrently recruited controls all of who 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 $GN\beta_3$), rectal compliance and pain sensation (with $5HTTLPR$). 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 $Pr_{1/2}$) and increased pain sensation ratings during rectal distension in those with $5HTTLPR$ LS/SS genotype relative to LL genotype. A decreased compliance may contribute to higher pain sensation ratings (6). However, since we observed increased compliance and higher pain sensation ratings, we conclude that the influence of $5HTTLPR$ LS/SS genotype on rectal compliance does not explain the increase in pain sensation observed with this genotype.
Therefore, this supports the hypothesis that 5HTTLPR 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 Figure 5 and data in Table III) constitutes a significant stimulus that overwhelms the hypothetical effect of the 5HTTLPR genotype proposed here. The LS/SS genetic variation would be expected to reduce re-uptake 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 5HTTLPR 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 TNBS (2,4,6-trinitrobenzene sulfonic 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. While 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. suggested there was an association between IBS-C or IBS-D and reduced SERT mRNA mucosal expression (16); 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 $\alpha_{2A}$ genotype on sensation of gas, urgency or pain is not altogether surprising since up-regulation of $\alpha_{2A}$ 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 pro-nociceptive changes in the ionic channel properties of primary afferent nociceptors. $\alpha_{2C}$ 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 $\alpha_{2C}$ receptor is too infrequent to allow us to explore its contribution to the endophenotype. On the other hand, $\alpha_{2A}$ 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 complexly 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, multi-factorial 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). While 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 irritable bowel syndrome.
REFERENCES


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<th>C-IBS</th>
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<th>M-IBS</th>
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<tr>
<td>Number (# male)</td>
<td>39 (0)</td>
<td>49 (0)</td>
<td>44 (3)</td>
<td>29 (0)</td>
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<td>Age, years</td>
<td>33.5 ± 1.6</td>
<td>38.4 ± 1.5</td>
<td>35.4 ± 1.6</td>
<td>37.2 ± 2.2</td>
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<td>BMI, kg/m²</td>
<td>25.1 ± 0.7</td>
<td>25.1 ± 0.4</td>
<td>28.9 ± 1.0</td>
<td>27.9 ± 0.8</td>
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<td>Anxiety score, HADS</td>
<td>3.2 ± 0.4</td>
<td>4.7 ± 0.5</td>
<td>5.0 ± 0.5</td>
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<td>Depression score, HADS</td>
<td>0.6 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>1.4 ± 0.2</td>
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<td>41.3 ± 1.0</td>
<td>47.6 ± 0.9</td>
<td>49.3 ± 1.2</td>
<td>48.8 ± 1.5</td>
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<td>SCL-90 General Severity Index T score</td>
<td>37.6 ± 1.3</td>
<td>44.0 ± 1.3</td>
<td>44.6 ± 1.5</td>
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<td>Somatic symptom score</td>
<td>0.3 ± 0.05</td>
<td>0.4 ± 0.04</td>
<td>0.5 ± 0.05</td>
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<td>Number taking SSRI (stable dose)</td>
<td>5</td>
<td>9</td>
<td>11</td>
<td>6</td>
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<tr>
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<td>25 (64.1)</td>
<td>21 (42.9)</td>
<td>24 (54.6)</td>
<td>12 (41.4)</td>
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<tr>
<td>α2A non-wildtype (GC/GG), n (%)</td>
<td>14 (35.9)</td>
<td>28 (57.1)</td>
<td>20 (45.4)</td>
<td>17 (58.6)</td>
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<td>α2C wildtype, n (%)</td>
<td>38 (97.4)</td>
<td>45 (91.8)</td>
<td>42 (95.4)</td>
<td>25 (86.2)</td>
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<td>α2C non-wildtype, n (%)</td>
<td>1 (2.6)</td>
<td>4 (8.2)</td>
<td>2 (4.6)</td>
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<td>GNβ3 wildtype (CC), n (%)</td>
<td>19 (48.7)</td>
<td>27 (55.1)</td>
<td>19 (43.2)</td>
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<td>20 (51.3)</td>
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<td>16 (41.0)</td>
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<td>23 (59.0)</td>
<td>28 (57.1)</td>
<td>28 (63.4)</td>
<td>22 (75.9)</td>
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^ Not considered in further analyses due to small numbers of non-wildtype (deleted)
Table II. Rectal Functions: Compliance, Sensation Ratings and Thresholds

<table>
<thead>
<tr>
<th>ENDPOINT †</th>
<th>HEALTH, n=39</th>
<th>IBS, n=122</th>
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<td></td>
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<tr>
<td>Pr ½, mmHg</td>
<td>(37) 12.3 ± 0.8</td>
<td>(118) 13.5 ± 0.4</td>
</tr>
<tr>
<td>Max Balloon Vol, mL</td>
<td>(37) 234 ± 16</td>
<td>(118) 238 ± 10</td>
</tr>
<tr>
<td><strong>Sensation Ratings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas @ 30 mmHg</td>
<td>(36) 63 ± 4</td>
<td>(111) 59 ± 2</td>
</tr>
<tr>
<td>Gas @ 36 mmHg</td>
<td>(33) 66 ± 5</td>
<td>(106) 62 ± 3</td>
</tr>
<tr>
<td>Pain @ 30 mmHg</td>
<td>(36) 56 ± 5</td>
<td>(111) 54 ± 3</td>
</tr>
<tr>
<td>Pain @ 36 mmHg</td>
<td>(33) 60 ± 4</td>
<td>(106) 57 ± 3</td>
</tr>
<tr>
<td>Urgency @ 30 mmHg</td>
<td>(36) 74 ± 3</td>
<td>(110) 73 ± 2</td>
</tr>
<tr>
<td>Urgency @ 36 mmHg</td>
<td>(33) 81 ± 2</td>
<td>(105) 76 ± 2</td>
</tr>
<tr>
<td><strong>Thresholds</strong> *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st Sensation</td>
<td>(37) 7.8 ± 0.6</td>
<td>(118) 8.1 ± 0.4</td>
</tr>
<tr>
<td>Gas</td>
<td>(37) 13.6 ± 1.5</td>
<td>(118) 13.9 ± 0.8</td>
</tr>
<tr>
<td>Urgency</td>
<td>(37) 17.2 ± 1.1</td>
<td>(117) 19.1 ± 0.9</td>
</tr>
<tr>
<td>Pain</td>
<td>(37) 28.8 ± 2.1</td>
<td>(118) 30.2 ± 1.1</td>
</tr>
</tbody>
</table>

† (n) mean ± SE

* (mean ± SE ignoring censored status)
**Table III. Relationship of Genotype to Satiation Volume, Gastric Volumes, Rectal Compliance, Sensation Ratings and Thresholds**

<table>
<thead>
<tr>
<th>ENDPOINT</th>
<th>α2A CC</th>
<th>α2A GC/GG</th>
<th>GNβ3 CC</th>
<th>GNβ3 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><strong>Satiation (MTV) volume, L</strong></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><strong>Fasting Gastric Volume, mL</strong></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>∆ Post-meal 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><strong>Compliance, Pr ½, mmHg</strong></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 @ 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 @ 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 @ 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 @ 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 @ 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 @ 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><strong>Threshold† 1st sensation, mmHg</strong></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>

* p <0.05 vs SLC6A4 LS/SS

† mean ± SE (includes censored values)
FIGURE LEGENDS

Figure 1 Experimental design for measuring rectal compliance and sensation using ascending method of limits and random order phasic distensions respectively. Anxiety/depression sensory ratings were recorded on each day of testing.

Figure 2 Association of GNβ3 genotype with gastric volumes; after adjusting for age, BMI, HAD score, somatization and group (IBS vs health) fasting gastric volume is significantly associated (p=0.03), and delta gastric volume borderline significantly associated (p=0.095).

Figure 3 Rectal compliance in health and IBS, based on 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).

Figure 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.