The *HTR3A* Polymorphism c. -42C>T Is Associated With Amygdala Responsiveness in Patients With Irritable Bowel Syndrome

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BACKGROUND & AIMS: 5-Hydroxytryptamine (5-HT) 3 receptor (5-HT_3R) antagonists are effective in treating patients with irritable bowel syndrome (IBS) and have anxiolytic effects. Their therapeutic effects are related, in part, to reducing amygdala engagement during expected visceral pain. A single nucleotide polymorphism in *HTR3A*, c.-42C>T;(C178T; rs1062613), is associated with altered reactivity of the amygdala during emotional face processing in healthy subjects (controls). We evaluated the influence of this single nucleotide polymorphism on amygdala reactivity to emotional faces and nonemotional stimuli in female patients with IBS and controls. **METHODS:** We measured brain responses during an affect-matching paradigm in 54 women (26 with IBS, 29 controls) using functional magnetic resonance imaging. We examined associations between *HTR3A* c.-42C>T genotype (C/C vs T carrier) and responses in amygdala and other regions of brain that expressed high levels of 5-HT_3R. **RESULTS:** The C/C genotype was associated with greater anxiety symptoms in patients with IBS and controls and increased activation of the amygdala under emotional and nonemotional conditions. Among patients with IBS, C/C genotype was associated with greater symptom ratings. A subset of IBS patients with the C/C genotype had increased amygdala responses to nonemotional stimuli, compared with other subjects with C/C genotype. **CONCLUSIONS: Regardless of diagnosis, the C/C genotype of the c.-42C>T polymorphism in *HTR3A*, compared with T carrier status, is associated with increased anxiety and amygdala responsiveness during emotional and nonemotional tasks. This polymorphism was associated with severity of IBS symptoms. Although this genotype is not sufficient for diagnosis of IBS, it is associated with severity of symptoms.**

Keywords: Serotonin; Genetics; Emotion; fMRI; Digestive Disorders.

Preclinical and clinical evidence supports an important role of the serotonin (5-hydroxytryptamine [5-HT]) signaling system in the modulation of the brain gut axis, and alterations in this signaling system may play a role in functional gastrointestinal disorders such as irritable bowel syndrome (IBS),[1] psychiatric disorders (anxiety, depression, and eating disorders[2]), and pregnancy-related nausea.[3] 5-HT_3R antagonists are one of the most effective treatments for patients with diarrhea-predominant IBS (IBS-D)[4–6] and also have shown effectiveness in comorbid chronic pain disorders, including functional dyspepsia and fibromyalgia.[7] Several clinical studies reported beneficial effects of 5-HT_3R antagonists in the treatment of anxiety,[8] which is highly comorbid with IBS, eating disorders, and fibromyalgia.[9] Animal studies support the concept that 5-HT_3R antagonists have anxiolytic effects by attenuating the response of emotional arousal circuits in the brain.[10–12] Even though the precise mechanism(s) underlying the effectiveness of the most widely tested 5-HT_3R antagonists (alosetron, cilansetron) in treating IBS-D symptoms remain incompletely understood, evidence supports both peripheral and central mechanisms of action.[4] For example, symptom improvement during alosetron treatment was associated with a reduction in amygdala activity during anticipation of abdominal pain,[13] consistent with a possible role of increased activity of amygdala-related emotional arousal circuits in the pathophysiology of IBS.[14]

5-HT_3Rs are widely distributed in the central and peripheral nervous systems, including the enteric nervous system.[15,16] Within the central nervous system, 5-HT_3Rs are found in amygdala, hippocampus, cingulate cortex, striatum, entorhinal frontal cortex, as well as brainstem and the superficial layers of the spinal cord.[17–19] 5-HT_3Rs are unique, representing the only ligand-gated cation

Abbreviations used in this paper: BA, Brodmann Area; fMRI, functional magnetic resonance image; GABA, γ-aminobutyric acid; HC, healthy controls; 5-HT, 5-hydroxytryptamine; IBS, irritable bowel syndrome; IBS-D, diarrhea-predominant irritable bowel syndrome; ME, match emotion; MF, match forms; PFC, prefrontal cortex; ROI, region of interest; SNP, single nucleotide polymorphism; SVC, small volume correction.

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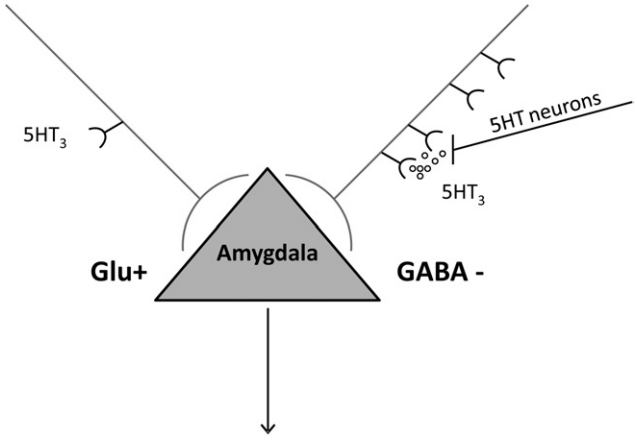

Figure 1. 5-HT_3R activation on GABAergic interneurons innervating the amygdala exerts an inhibitory influence on amygdala activity, whereas those on excitatory glutaminergic interneurons will exert an excitatory influence.

channel among 5-HT receptors, which are primarily expressed presynaptically.[20,21] The presynaptic expression of 5-HT_3Rs on nerve endings is consistent with their physiological role in the release of neurotransmitters such as dopamine, cholecystokinin, glutamate, acetylcholine, and γ-aminobutyric acid (GABA).[22] Consequently, both excitatory and inhibitory effects of 5-HT_3R activation have been reported that may be mediated by activation of excitatory and inhibitory interneurons, respectively.[23] For example, 5-HT_3R activation on GABAergic interneurons innervating the amygdala exert an inhibitory influence on amygdala activity, whereas those on excitatory glutaminergic interneurons have the opposite effect[23] (Figure 1).

There is considerable molecular diversity within the 5-HT_3R family resulting from variable composition of 5 different subunits (5-$HT_{3A–E}$), with differential expression of subunits in different regions of the nervous system.[23] Several polymorphisms of the 5-HT_{3A}R, 5-HT_{3B}R, 5-HT_{3C}R, and 5-HT_{3E}R encoding genes (*HTR3A*, *B*, *C*, and *E*) have been reported to be associated with gastrointestinal disorders (including IBS),[24] pregnancy-related nausea,[3] and several psychiatric conditions (including anxiety, depression, and eating disorders).[2,25,26] For example, the novel *HTR3E* 3′-untranslated region variant c.*76G>A has been shown to be associated with a diagnosis of IBS-D in female patients in two independent samples, suggesting a possible role of this variant in intestinal fluid handling.[24]

The relatively common variant c.-42C>T (minor allele frequency, 0.15–0.23) within the 5′ untranslated region of the *HTR3A* gene is associated with increased 5-HT_{3A} subunit expression in vitro.[24,26] The less common T allele, vs the C allele, is associated with reduced amygdala and prefrontal cortical activation during a face-recognition task and may be associated with the personality trait of lower harm avoidance in women.[27,28] These findings are consistent with T-allele–related increased expression of 5-HT_3Rs on inhibitory GABAergic interneurons, resulting in greater inhibition of the amygdala.

We aimed to examine, in female IBS patients and healthy controls (HCs), the impact of *HTR3A* c.-42C>T on responses in 5-HT_3R–rich brain regions during an affect-matching paradigm known to reliably activate the amygdala.[29] Based on the evidence summarized earlier, and the concept of increased engagement of amygdala-related emotional arousal circuits in IBS pathophysiology,[14] our main hypothesis was that subjects carrying the C/C genotype of the *HTR3A* single nucleotide polymorphism (SNP) c.-42C>T would show greater responses of the amygdala, and possibly greater IBS severity.

Materials and Methods

Subjects

A sample of 26 female IBS patients and 29 female healthy controls (demographics are presented in Table 1) participated in functional magnetic resonance image (fMRI) studies of emotional reactivity and provided saliva samples for DNA analyses. Subjects were recruited through the University of California Los Angeles Digestive Disease Clinic and from community advertisements. The diagnosis of IBS was confirmed using Rome II criteria during a clinical examination by a gastroenterologist or nurse practitioner experienced in functional gastrointestinal disorders.[30] Nineteen percent of IBS patients rated their usual symptoms as moderate (cannot be ignored but does not affect your lifestyle), 62% as severe (affects your lifestyle), and 19% as very severe (markedly affects your lifestyle). Control subjects received medical examinations to confirm absence of functional pain disorders. All subjects were free of current or past psychiatric illness, substance abuse disorder, and major medical or neurologic conditions. No subjects took medications

Table 1. Clinical Characteristics

	C/C	T carrier
N		
Control	15	14
IBS	16	10
Age		
Control	27.2 (3.1)	39.93 (3.7)
IBS	32.06 (2.5)	31.00 (3.6)
Anxiety		
Control	4.5 (0.5)	2.6 (0.8)
IBS	5.8 (0.9)	4.5 (0.9)
IBS		
Bowel habit	6 IBS-C, 8 IBS-D, 2 IBS-A	2 IBS-C, 3 IBS-D, 5 IBS-A
Overall symptom severity	13.3 (1.2)	9.6 (1.0)
Bloating severity	13.1 (1.1)	7.7 (1.9)
Abdominal pain severity	11.3 (1.5)	9.0 (1.2)

NOTE. Numbers in parentheses refer to standard errors.
IBS-C, constipation predominate; IBS-A, alternating bowel habit.

for 30 days before scanning. The University of California Los Angeles Institutional Review Board approved all studies; informed consent was obtained from all subjects.

Genotyping

DNA was extracted at the University of California Los Angeles Biological Samples Processing Core. Samples for DNA isolation were collected using the Oragene DNA Self-Collection Kit (DNA Genotek, Inc, Ottawa, Canada). DNA obtained using this kit is comparable in quality and quantity with DNA extracted from blood.[31] SNP genotyping was performed with the KASPar assay system (KBiosciences, Ltd, Hoddesdon, UK) as recommended by the manufacturer. The primers used for *HTR3A* c.-42C>T were as follows: rs1062613_ALC: 5′-GAAGGTGACCAAGTTCATGCTGCCTCCGAGTGCTCAGGG-3′; rs1062613_ALT: 5′-GAAGGTCGGAGTCAACGGATTGTGCCTCCGAGTGCTCAGGA-3′; and rs1062613_C1: 5′-AGGTTGGCAGAGGGCAGGCAA-3′. An initial 15-minute cycle at 94°C was followed by 20 cycles consisting of 10 seconds at 94°C, 5 seconds at 57°C, and 10 seconds at 72°C; 23 cycles consisting of 10 seconds at 94°C, 20 seconds at 57°C, and 40 seconds at 72°C; and a cool down at 10°C. After the thermal cycling, results were analyzed using the fluorescence plate reader TaqMan 7500 system (Applied Biosystems, Foster City, CA).

Study Design

Before scanning, IBS patients rated the following: (1) overall severity of their gastrointestinal symptoms, (2) severity of abdominal pain, and (3) severity of bloating on a numeric rating scale of 0 (no symptoms) to 20 (the most intense symptoms imaginable). All subjects completed the Hospital Anxiety and Depression scale, a measure of current anxiety symptoms validated for nonpsychiatric samples.[32]

Subjects completed 2 runs of an emotional reactivity task (adapted from Hariri et al[29]) consisting of 2 conditions: match emotion (ME) and match forms (MF). During the ME condition, subjects viewed a target face[33] depicting an angry or fearful expression and were asked to select 1 of 2 other faces that expressed the same emotion. In the ME task, subjects tend to match the emotional faces based on perceptual characteristics, such as a furrowed brow.[29] The MF condition is a neutral control task in which subjects perform a similar perceptual decision (match forms), but no emotional stimuli are presented. During the MF condition, subjects viewed a circular-shaped target (approximately the same size as a human face) and were asked to select 1 of 2 other shapes that best matched the target. Traditionally, the difference in activity during the emotional condition compared with the neutral condition (ME−MF) has been considered a measure of emotional reactivity.[29]

Stimuli were presented in randomized sequences, counterbalanced across runs. Each condition was presented as a block of 6 images, with each image presented for 3 seconds, for a total block length of 18 seconds. In each run, 4 blocks of ME and 4 blocks of MFs were presented randomly. Subjects completed 2 runs, thus a total of 48 images were presented in the ME condition and 48 images were presented in the MF condition. An instruction cue was presented for 3 seconds before each block and a rest period of 6 seconds followed each block. Subjects were presented with additional facial stimuli as part of an emotional modulation task; however, the current study involves the results of the emotional reactivity task only.

fMRI Procedures

fMRI was performed using a 3.0-T MRI scanner (Siemens Trio; Siemens, Erlangen, Germany). A high-resolution structural image was acquired from each subject with a magnetization-prepared rapid acquisition gradient-echo sequence, repetition time of 2300 ms, echo time of 2.85 ms, 256 slices, 160*240 matrix, 1^3 mm voxel size. Functional blood oxygen-level dependent images were acquired (repetition time of 3000 ms, echo time of 28 ms, flip angle of 90°, 38 slices, slice thickness of 3 mm) while subjects completed 2 runs of the emotional reactivity task. Stimuli were presented via MRI-compatible goggles using Superlab 4.0 software (Cedrus Corp, San Pedro, CA). Subjects responded using an MRI-compatible button box by pressing 1 of 2 buttons with the right hand.

Data Analysis

The first 2 volumes were discarded to allow for stabilization of the magnetic field. The remaining functional images were slice-time– and motion corrected, spatially normalized to the Montreal Neurological Institute (MNI) template, and spatially smoothed with a 8-mm^3 Gaussian kernel using SPM5 (Welcome Department of Cognitive Neurology, London, UK). Although the amygdala was the primary region of interest (ROI), additional ROIs were chosen a priori and consisted of brain areas previously associated with 5-HT_3R distribution.[17–19] ROIs for the amygdala, caudate, hippocampus, insula, anterior cingulate, and medial prefrontal cortex (PFC) were created using the Wake Forest University (Winston-Salem, North Carolina) PickAtlas toolbox in SPM5.[34–37] Functional data were analyzed using the general linear model within SPM5. For each individual subject, fixed-effect analyses were performed comparing the following: (1) ME vs an implicit baseline, (2) MF vs an implicit baseline, and (3) ME vs MF (ME-MF). Parameter estimates for ME-MF were considered a measure of emotional reactivity.[29] We included analyses of brain responses during ME and MF conditions (considered individually) to provide additional information on potential group differences in the specificity of brain responsiveness. Random-effect analyses were performed to

examine genotype and diagnosis effects with age of the subject entered as a covariate. Small volume correction (SVC) for ROIs was applied using SPM5 family-wise error algorithm and a volume-corrected probability value of less than 0.05 was considered significant. A priori power analyses conducted in G*Power 3.1.2 (www.psycho.uni-duesseldorf.de/aap/projects/gpower/) indicated that 10 subjects per group were required to provide adequate power ($1 - \beta = 0.80$) to detect an effect size difference ($d = 1.20$, $z = 2.4$) in brain activity commonly seen in our research studies, using a one-sample t test.

The association of genotype with diagnosis was analyzed by chi-square and risk assessment tests; the genetic impact on anxiety and symptom ratings was analyzed by analysis of variance using PASW 17 (SPSS, Inc; Chicago, IL).

Results

Clinical Characteristics

HTR3A polymorphism. Fifteen of 29 HCs and 16 of 26 IBS patients were homozygous for the C allele, 9 HCs and 7 patients carried the heterozygous C/T genotype, and 5 HCs and 3 patients carried the homozygous T/T genotype. The c.-42C>T SNP had been found previously to be associated with IBS-D in a UK sample applying a minor allele dominant model.[24] Thus, subjects carrying a T allele (C/T or T/T) were combined into a single group (T carriers) for analyses.

A *HTR3A* genotype (C/C, T carrier) × diagnosis (HC, IBS) analysis of variance revealed a significant interaction of diagnosis and T-carrier status on anxiety symptoms, with IBS patients reporting higher anxiety ratings, and T carriers (IBS and HCs combined) reporting lower anxiety ratings ($F(1,51) = 4.20$, $P = .046$; $F(1,51) = 4.37$, $P = .042$, respectively). Among IBS patients, T carriers had significantly lower overall symptom severity and abdominal discomfort (bloating) ratings ($t(24) = 2.102$, $P = .046$, $t(24) = 2.654$, $P = .014$, respectively). However, T carriers with IBS did not differ in abdominal pain ratings ($P > .05$). Means and standard errors are displayed in Table 1. Chi-square tests and risk estimates indicated no significant association between T-carrier status and IBS diagnosis ($P > .05$).

Emotional Reactivity (ME-MF) at the Whole Brain Level

Activity during ME relative to MF (ME-MF; with age entered as a covariate) was first examined at the whole brain level to determine if regions differentially activated in response to emotional faces compared with neutral visual stimuli were similar to previous studies of emotional reactivity. Areas that were significantly activated ($P < .05$, family-wise error, extent ≥ 30 voxels) across all subjects included right amygdala, bilateral visual cortex (Brodmann Area [BA] 17/18/19), bilateral fusiform gyrus (BA 37), bilateral thalamus, bilateral dorsolateral and ventrolateral PFC (BA 8/9/45/46/47), bilateral BA 7, and right BA 22/41 (Table 2). Areas that were significantly deactivated ($P < .05$, family-wise error, extent ≥ 30 voxels) during ME relative to MF included bilateral medial PFC (BA 24/32), bilateral insula, bilateral posterior cingulate cortex (BA 23/31), and left BA 39 (Table 2). These results are consistent with previous studies using a similar emotional reactivity paradigm.[29,38]

HTR3A Effects on Brain Responses in Specific Regions

An ROI analysis was conducted to examine diagnosis and *HTR3A* genotype effects on activity of the amygdala during a neutral visual control task (MF) and during an emotional task (ME) as well as effects on emotional reactivity (ME-MF). We hypothesized greater amygdala responses in C/C genotype subjects. In addition, we performed exploratory analyses of potential diagnosis and *HTR3A* genotype effects on activity of other 5-HT_3R–rich brain regions.

MF. During MF, IBS subjects relative to HCs had significantly greater activity in the left amygdala whereas C/C genotype subjects (IBS and HCs combined) had greater activity in bilateral amygdala (Figure 2) relative to T carriers (Table 2; $P < .05$, SVC). No significant interactions in amygdala responses were found. Exploratory analyses showed significantly greater activity in the left caudate and hippocampus in IBS subjects relative to HCs and greater activity in the right hippocampus in C/C genotype subjects (IBS and HCs combined) relative to T carriers (Table 2; $P < .05$, SVC); however, only the left hippocampus remained formally significant with Bonferroni correction for multiple comparisons.

ME. During ME, C/C genotype subjects (IBS and HCs combined) had greater activity in the right amygdala (Figure 2; Table 2; $P < .05$, SVC). No significant diagnosis effects or interactions in amygdala responses were found. Exploratory analyses showed significantly greater activity in the left caudate and hippocampus of IBS subjects relative to HCs (Figure 2; Table 2; $P < .05$, SVC), however, only the left hippocampus remained formally significant with Bonferroni correction for multiple comparisons.

ME-MF. Although group differences existed in amygdala responses during ME and MF considered individually (see earlier), no significant group differences or interactions in amygdala emotional reactivity were found. Exploratory analyses showed that HCs relative to IBS patients had greater differential activity in the right insula during ME relative to MF condition (Table 2; $P < .05$, SVC). Also, T carriers relative to C/C genotype subjects (IBS and HCs combined) had greater differential activity in the left caudate and right hippocampus (Table 2; $P < .05$, SVC). However, none of these results remained formally significant with Bonferroni correction for multiple comparisons.

Table 2. Peak MNI Coordinates of Significant Activations and Group Differences

	Hemisphere	Region	Cluster size	*P*	T	X, mm	Y, mm	Z, mm
Whole brain								
All subjects								
ME-MF	Bilateral	BA 17/18/19/37	11576	<.001	18.87	22	-92	-10
				<.001	16.49	−18	−92	−6
	Bilateral	Thalamus	919	<.001	10.62	20	−30	−2
				<.001	10.39	−20	−30	−2
	Right	Amygdala	59	<.001	7.90	20	−6	−14
	Right	BA 45/46/47	2747	<.001	10.45	50	20	22
	Left	BA 9/45/47	2020	<.001	10.40	−40	8	28
	Left	BA 7	240	<.001	7.35	−26	−58	44
	Right	BA 22/41	351	<.001	7.23	54	−42	6
	Right	BA 7	171	<.001	7.16	34	−58	42
	Bilateral	BA 8	182	.003	6.27	−4	16	50
				.006	5.99	4	18	50
MF-ME	Bilateral	BA 24/32	2145	<.001	11.79	−6	34	−4
				<.001	9.54	6	34	−4
	Right	Insula	1171	<.001	7.38	42	−18	18
	Left	Insula	742	<.001	7.58	−44	−22	6
	Left	BA 39	175	<.001	7.57	−42	−72	36
	Left	BA 23	136	<.001	7.54	−8	−56	14
	Right	BA 31	369	<.001	7.35	4	−28	44
ROI analyses								
MF								
IBS>HC	Left	Amygdala	104	.043	2.90	−22	−10	−12
	Left	Caudate	65	.023	3.74	−26	−40	4
	Left	Hippocampus	156	.005	3.85	−26	−40	2
C/C>T carrier	Left	Amygdala	81	.032	3.03	−26	−4	−26
	Right	Amygdala	72	.012	3.46	30	−6	−22
	Right	Hippocampus	67	.031	3.13	30	−8	−22
ME								
IBS>HC	Left	Caudate	59	.015	3.94	−26	−40	4
	Left	Hippocampus	69	.005	3.95	−26	−38	2
C/C>T carrier	Right	Amygdala	59	.03	3.12	30	−6	−20
ME-MF:								
HC>IBS	Right	Insula	1257	.015	4.28	42	−4	16
T carrier>C/C	Left	Caudate	142	.022	3.83	−8	2	18
	Left	Hippocampus	61	.046	3.05	−26	−24	−12
	Right	Hippocampus	161	.014	3.52	32	−22	−14
C/C genotype Median split								
MF								
IBS Low > IBS High	Right	Amygdala	135	.006	4.04	26	−2	−20
IBS Low > HC	Right	Amygdala	138	.013	3.69	26	−2	−20

Parameter estimates for emotional reactivity (ME-MF) of the right amygdala were extracted for each subject using the Marsbar toolbox (http://marsbar.sourceforge.net/).[39] As shown in Figure 3, right amygdala reactivity estimates varied greatly among IBS patients with C/C genotype. Bartlett's test for homogeneity of variances showed significant differences in variance ($\chi^2(3) = 9.62$, $P = .022$) such that IBS patients with C/C genotype had significantly more variance than IBS T carriers and HCs with C/C genotype ($\chi^2(1) = 5.22$, $P = .022$; $\chi^2(1) = 6.58$, $P = .01$, respectively). The greater variance appeared to be owing to a subgroup of IBS patients with C/C genotype with unusually low differential activity during ME relative to MF. Therefore, additional analyses were performed to determine if this subgroup had higher amygdala responses during MF and/or lower amygdala responses during ME compared with other subjects with C/C genotype.

C/C genotype median split. An ROI analysis using a median split was performed, comparing IBS patients with C/C genotype and below median reactivity (IBS Low; n = 8) with: (1) IBS patients with C/C genotype and above median reactivity (IBS High; n = 8) and (2) HC with C/C genotype (n = 15). Among subjects with C/C genotype, IBS subjects with low differential activity had significantly greater activity in the right amygdala during MF compared with both IBS and HCs with above median reactivity (Table 2). No significant differences were found during ME. Also, no significant differences between IBS patients with C/C genotype and above median reactivity and HC with C/C genotype were found.

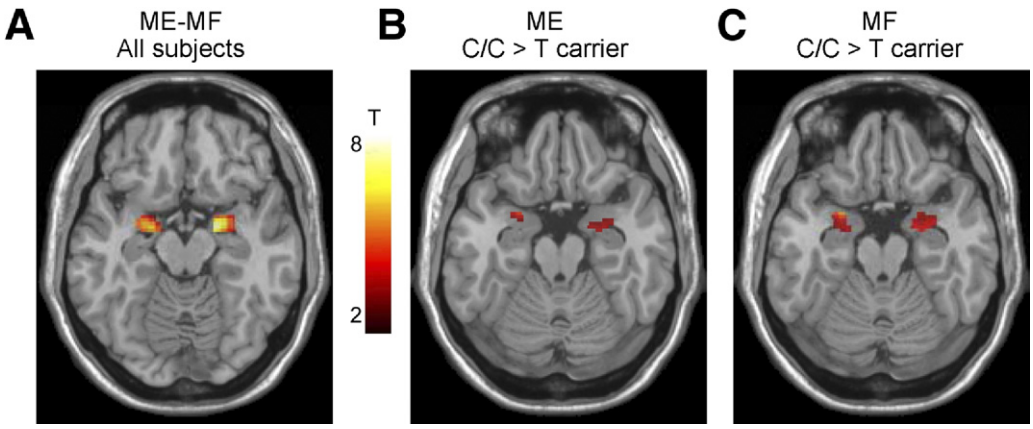

Figure 2. (*A*) The emotional reactivity paradigm activated the amygdala. C/C genotype subjects, regardless of diagnosis, displayed increased amygdala responsiveness during the (*B*) ME condition and during the (*C*) MF condition.

Discussion

We used a validated emotional reactivity paradigm to identify differences between IBS patients and HCs in emotional arousal–related brain responses. When contrasting the brain's response to the negative emotional faces with the response to neutral geometric forms (ME-MF), consistent activations in amygdala, thalamus, and lateral prefrontal and parietal cortices, and deactivations in medial PFC, insula, and posterior cingulate cortex were seen. C/C genotype, regardless of diagnosis, was associated with significantly greater anxiety and greater amygdala responses to emotional facial as well as neutral visual stimuli when brain responses during each condition were examined individually (ME and MF). In patients, the C/C genotype was associated with higher IBS symptom ratings, and a subset of IBS patients with C/C genotype had even greater amygdala responses during the neutral stimulus (eg, subjects with usually low differential activity during ME relative to MF), compared with other subjects with C/C genotype (eg, IBS/HC with a normal pattern of responses). Thus, even though the CC genotype conferred altered amygdala responsiveness and associated anxiety ratings across both IBS and HCs, there was also an interaction of this genotype with IBS diagnosis.

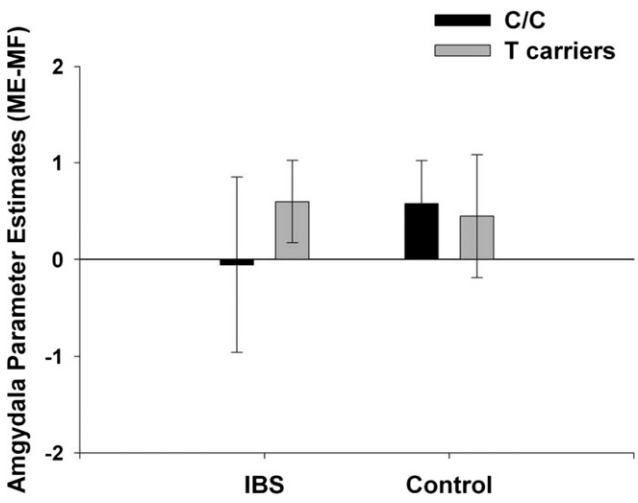

Figure 3. Mean and standard deviation for right amygdala emotional reactivity (defined as $\beta_{ME\text{-}MF}$) is plotted for healthy controls and IBS patients by *HTR3A* c.-42C>T genotype (C/C vs T carrier). A subset of IBS subjects with C/C genotype had an unusual pattern of amygdala response.

C/C genotype across groups showed greater amygdala responses to both emotional and nonemotional stimuli when compared with T carriers. This indiscriminate pattern of response suggests a generalized hyper-responsiveness of the amygdala in C/C genotype subjects. Indiscriminate or generalized increased amygdala responsiveness to emotional facial stimuli after acute experimental stress has been reported recently.[40] The investigators suggested that a shift of amygdala function occurred toward heightened sensitivity with lower levels of specificity, consistent with a state of hypervigilance under stress. Our findings suggest that the C/C genotype predisposes individuals to such indiscriminate hypervigilance. Consistent with this interpretation, Iidaka et al[28] showed increased amygdala and PFC responses to pictures of neutral faces (relative to pictures of houses) in HCs with C/C genotype compared with C/T genotype subjects. Despite a difference in paradigms, the current results are similar in that subjects with C/C genotype appear to be hyper-responsive to facial stimuli compared with T carriers. However, in the current study, subjects with C/C genotype were not specifically hyper-responsive to facial stimuli because they showed heightened amygdala responses when processing both emotional faces and nonemotional geometric forms. In other words, they showed a generalized amygdala hyper-responsiveness regardless of the emotional content of the images. Both HCs and IBS with the C/C genotype may have increased sustained or tonic activation of the amygdala, consistent with their greater anxiety symptoms relative to T-carrier subjects.

Further analyses revealed a subgroup of IBS patients with C/C genotype that had unusually low differential amygdala activity during ME relative to MF (Figure 3). These subjects failed to discriminate between emotional and neutral stimuli owing to heightened amygdala responses during the nonemotional condition. These findings suggest an interaction between IBS diagnosis and C/C genotype. It remains to be determined if the unusual response pattern of the amygdala to neutral and emotional stimuli represents an endophenotype[41] that is associated with the *HTR3A* SNP, or perhaps is related to increased stress perceived by some IBS patients during the experiment.

The T allele is related to an increase of *HTR3A* expression compared with the C allele.[24,26] Greater presynaptic expression of *HTR3A* on inhibitory GABAergic input to the amygdala is expected to result in greater GABAergic inhibition of the amygdala during serotonergic stimulation.[42,43] Increased inhibitory GABAergic function in the amygdala via benzodiazepines,[44] microinfusion of $GABA_A$-receptor agonists,[45] or neuron-rich grafts[46] has been associated with decreased anxiety. These results suggest T carriers have enhanced amygdala 5-HT_{3A} expression and GABAergic function associated with decreased anxiety. Both preclinical and clinical evidence supports a role for 5-HT_3Rs in anxiety. For example, results from rodent studies have led to the consensus that 5-HT_3R antagonists have anxiolytic effects by blocking limbic hyperactivity response.[10–12] Clinical studies reported on beneficial effects of 5-HT_3R antagonists in the treatment of anxiety.[47,48] 5-HT_3R knockout mice have shown differences between sexes in 5-HT_3 regulation of depression- and anxiety-related behaviors.[49] Future studies will be required to determine if differences in sex exist in the association between C/C genotype and generalized amygdala responsiveness, anxiety, and IBS severity.

Limitations

IBS patient recruitment included all bowel habit types, and no relationship between bowel habit and genotype/amygdala response patterns was found in this relatively small sample (data not shown). Future studies with larger sample sizes for each bowel habit subtype are needed to adequately examine the specificity of the relationship between *HTR3A* c.-42C>T polymorphism and amygdala responsiveness in terms of bowel habit.

Summary and Future Directions

By using a validated emotional reactivity paradigm, unrelated to the gastrointestinal system, we found significant correlations of the *HTR3A* c.-42C>T polymorphism with amygdala responsiveness, anxiety, and IBS symptom severity in a relatively small sample. The study supports the important role of 5-HT_3Rs in the modulation of emotional arousal circuits that previously have been shown to be related to 5-HT_3R antagonist-mediated (alosetron) IBS symptom improvement.[13] These findings suggest that although this gene polymorphism is not essential for a diagnosis of IBS, it influences symptom severity via greater engagement of amygdala-related emotional arousal circuits. This is consistent with previous reports showing an important role of anxiety in symptom severity in IBS[50] and in functional dyspepsia.[51]

The differential responsiveness of the amygdala based on genotype could underlie differences in responsiveness of individual IBS patients to 5-HT_3R antagonists, as well as other centrally directed therapies. For example, only about 40% of patients were identified as responders in clinical trials with the 5-HT_3R antagonist alosetron.[6] Alosetron's effectiveness in reducing symptoms in nonconstipated IBS patients has been associated with reduced amygdala responsiveness during nonaversive and aversive conditions.[13] The current study shows an effect of *HTR3A* c.-42C>T polymorphism on amygdala reactivity to emotional and nonemotional stimuli. Additional research is needed to examine the relationship between emotional reactivity and visceral pain reactivity, and to directly examine the relationship between *HTR3A* c.-42C>T polymorphism, amygdala reactivity, and response to 5-HT_3R antagonists. Patients with C/C genotype appear to have up-regulated amygdala activity, thus they may be more resistant to treatment by 5-HT_3R antagonists.[52] In general, subtyping of IBS patients based on gene variants of central receptors modulating emotional arousal may improve the outcome of future clinical trials.

References

1. Bradesi S, Mayer EA. Novel therapeutic approaches in IBS. Curr Opin Pharmacol 2007;7:598–604.
2. Hammer C, Kapeller J, Endele M, et al. Functional variants of the serotonin receptor type 3A and B gene are associated with eating disorders. Pharmacogenet Genomics 2009;19:790–799.
3. Goecke TW, Ekici AB, Niesler B, et al. Two naturally occurring variants of the serotonin receptor gene HTR3C are associated with nausea in pregnancy. Acta Obstet Gynecol Scand 2010;89:7–14.
4. Mayer EA, Bradesi S. Alosetron and irritable bowel syndrome. Expert Opin Pharmacother 2003;4:2089–2098.
5. Johanson JF. Options for patients with irritable bowel syndrome: contrasting traditional and novel serotonergic therapies. Neurogastroenterol Motil 2004;16:701–711.
6. Chang L, Ameen VZ, Dukes GE, et al. A dose-ranging, phase II study of the efficacy and safety of alosetron in men with diarrhea-predominant IBS. Am J Gastroenterol 2005;100:115–123.
7. Faerber L, Drechsler S, Ladenburger S, et al. The neuronal 5-HT3 receptor network after 20 years of research—evolving concepts in management of pain and inflammation. Eur J Pharmacol 2007;560:1–8.
8. Rajkumar R, Mahesh R. The auspicious role of the 5-HT3 receptor in depression: a probable neuronal target? J Psychopharmacol 2010;24:455–469.
9. Aina Y, Susman JL. Understanding comorbidity with depression and anxiety disorders. J Am Osteopath Assoc 2006;106:S9–S14.

10. Griebel G. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol Ther 1995;65:319–395.
11. Millan MJ. The neurobiology and control of anxious states. Prog Neurobiol 2003;70:83–244.
12. Lecrubier Y, Puech AJ, Azcona A, et al. A randomized double-blind placebo-controlled study of tropisetron in the treatment of outpatients with generalized anxiety disorder. Psychopharmacology (Berl) 1993;112:129–133.
13. Berman SM, Chang L, Suyenobu B, et al. Condition-specific deactivation of brain regions by 5-HT3 receptor antagonist Alosetron. Gastroenterology 2002;123:969–977.
14. Labus JS, Naliboff BN, Fallon J, et al. Sex differences in brain activity during aversive visceral stimulation and its expectation in patients with chronic abdominal pain: a network analysis. Neuroimage 2008;41:1032–1043.
15. Kilpatrick GJ, Jones BJ, Tyers MB. Identification and distribution of 5-HT3 receptors in rat brain using radioligand binding. Nature 1987;330:746–748.
16. Fozard JR. Neuronal 5-HT receptors in the periphery. Neuropharmacology 1984;23:1473–1486.
17. Laporte AM, Koscielniak T, Ponchant M, et al. Quantitative autoradiographic mapping of 5-HT3 receptors in the rat CNS using [125I]iodo-zacopride and [3H]zacopride as radioligands. Synapse 1992;10:271–281.
18. Tecott LH, Maricq AV, Julius D. Nervous system distribution of the serotonin 5-HT3 receptor mRNA. Proc Natl Acad Sci U S A 1993; 90:1430–1434.
19. Morales M, Battenberg E, Bloom FE. Distribution of neurons expressing immunoreactivity for the 5HT3 receptor subtype in the rat brain and spinal cord. J Comp Neurol 1998;402:385–401.
20. Maricq AV, Peterson AS, Brake AJ, et al. Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel. Science 1991;254:432–437.
21. Yakel JL, Jackson MB. 5-HT3 receptors mediate rapid responses in cultured hippocampus and a clonal cell line. Neuron 1988;1: 615–621.
22. Hannon J, Hoyer D. Molecular biology of 5-HT receptors. Behav Brain Res 2008;195:198–213.
23. Barnes NM, Hales TG, Lummis SC, et al. The 5-HT3 receptor—the relationship between structure and function. Neuropharmacology 2009;56:273–284.
24. Kapeller J, Houghton LA, Monnikes H, et al. First evidence for an association of a functional variant in the microRNA-510 target site of the serotonin receptor-type 3E gene with diarrhea predominant irritable bowel syndrome. Hum Mol Genet 2008;17:2967–2977.
25. Walstab J, Rappold G, Niesler B. 5-HT(3) receptors: role in disease and target of drugs. Pharmacol Ther 2010;128:146–169.
26. Niesler B, Weiss B, Fischer C, et al. Serotonin receptor gene HTR3A variants in schizophrenic and bipolar affective patients. Pharmacogenetics 2001;11:21–27.
27. Melke J, Westberg L, Nilsson S, et al. A polymorphism in the serotonin receptor 3A (HTR3A) gene and its association with harm avoidance in women. Arch Gen Psychiatry 2003;60:1017–1023.
28. Iidaka T, Ozaki N, Matsumoto A, et al. A variant C178T in the regulatory region of the serotonin receptor gene HTR3A modulates neural activation in the human amygdala. J Neurosci 2005; 25:6460–6466.
29. Hariri AR, Bookheimer SY, Mazziotta JC. Modulating emotional responses: effects of a neocortical network on the limbic system. NeuroReport 2000;11:43–48.
30. Thompson WG, Longstreth GF, Drossman DA, et al. Functional bowel disorders and functional abdominal pain. Gut 1999; 45(Suppl 2):II43–II47.
31. Reynolds JD, Do TT, Hongo DB, et al. Comparison of high density genotyping results from saliva and blood samples on Affymetrix GeneChip® GenomeWide SNP 6.0 arrays. Annual meeting of the American Society of Human Genetics, San Diego, CA, 2007.
32. Zigmond As SRP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983;67:361–370.
33. Ekman P, Friesen WV. Constants across cultures in the face and emotion. J Pers Soc Psychol 1971;17:124–129.
34. Maldjian JA, Laurienti PJ, Kraft RA, et al. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage 2003;19:1233–1239.
35. Maldjian JA, Laurienti PJ, Burdette JH. Precentral gyrus discrepancy in electronic versions of the Talairach atlas. Neuroimage 2004;21:450–455.
36. Lancaster JL, Rainey LH, Summerlin JL, et al. Automated labeling of the human brain: a preliminary report on the development and evaluation of a forward-transform method. Hum Brain Mapp 1997;5:238–242.
37. Lancaster JL, Woldorff MG, Parsons LM, et al. Automated Talairach atlas labels for functional brain mapping. Hum Brain Mapp 2000;10:120–131.
38. Payer DE, Lieberman MD, Monterosso JR, et al. Differences in cortical activity between methamphetamine-dependent and healthy individuals performing a facial affect matching task. Drug Alcohol Depend 2008;93:93–102.
39. Brett M, Anton JL, Valabregue R, et al. Region of interest analysis using an SPM toolbox. Eighth International Conference on Functional Mapping of the Human Brain. Sendai, Japan, 2002.
40. van Marle HJ, Hermans EJ, Qin S, et al. From specificity to sensitivity: how acute stress affects amygdala processing of biologically salient stimuli. Biol Psychiatry 2009;66:649–655.
41. Labus JS, Tillisch K, Coveleskie K, et al. Functional neuroimaging paradigm identifies amygdala responsiveness as potential neurobiological endophenotype in IBS. Joint International Neurogastroenterology and Motility Meeting. Boston, Massachusetts, 2010.
42. Turner TJ, Mokler DJ, Luebke JI. Calcium influx through presynaptic 5-HT3 receptors facilitates GABA release in the hippocampus: in vitro slice and synaptosome studies. Neuroscience 2004; 129:703–718.
43. Koyama S, Matsumoto N, Kubo C, et al. Presynaptic 5-HT3 receptor-mediated modulation of synaptic GABA release in the mechanically dissociated rat amygdala neurons. J Physiol 2000; 529:373–383.
44. Kajimura N, Nishikawa M, Uchiyama M, et al. Deactivation by benzodiazepine of the basal forebrain and amygdala in normal humans during sleep: a placebo-controlled [15O]H2O PET study. Am J Psychiatry 2004;161:748–751.
45. Sanders SK, Shekhar A. Regulation of anxiety by GABAA receptors in the rat amygdala. Pharmacol Biochem Behav 1995;52: 701–706.
46. Cunningham MG, Connor CM, Carlezon WA Jr, et al. Amygdalar GABAergic-rich neural grafts attenuate anxiety-like behavior in rats. Behav Brain Res 2009;205:146–153.
47. Dolnak DR. Treating patients for comorbid depression, anxiety disorders, and somatic illnesses. J Am Osteopath Assoc 2006; 106:S1–S8.
48. Harmer CJ, Reid CB, Ray MK, et al. 5HT(3) antagonism abolishes the emotion potentiated startle effect in humans. Psychopharmacology (Berl) 2006;186:18–24.
49. Bhatnagar S, Nowak N, Babich L, et al. Deletion of the 5-HT3 receptor differentially affects behavior of males and females in the Porsolt forced swim and defensive withdrawal tests. Behav Brain Res 2004;153:527–535.
50. Labus JS, Bolus R, Chang L, et al. The Visceral Sensitivity Index: development and validation of a gastrointestinal symptom-specific anxiety scale. Aliment Pharmacol Ther 2004;20:89–97.

51. Van Oudenhove L, Vandenberghe J, Geeraerts B, et al. Relationship between anxiety and gastric sensorimotor function in functional dyspepsia. Psychosom Med 2007;69:455–463.
52. Jarcho JM, Chang L, Berman M, et al. Neural and psychological predictors of treatment response in irritable bowel syndrome patients with a 5-HT3 receptor antagonist: a pilot study. Aliment Pharmacol Ther 2008;28:344–352.

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Conflicts of interest

The authors disclose no conflicts.

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