IRRITABLE BOWEL SYNDROME

Association of distinct α2 adrenoceptor and serotonin transporter polymorphisms with constipation and somatic symptoms in functional gastrointestinal disorders

H J Kim, M Camilleri, P J Carlson, F Cremonini, I Ferber, D Stephens, S McKinzie, A R Zinsmeister, R Urrutia

Background: The role of genetics in the phenotypic manifestations of irritable bowel syndrome (IBS) is unclear. Our aims were: (1) to compare the prevalence of polymorphisms of alpha 2 (α2) adrenoceptors, norepinephrine transporter, and serotonin transporter protein (soluble carrier protein member 4 (SLC6A4)) promoter in patients with lower functional gastrointestinal disorders (FGID) and in healthy controls; and (2) to test associations of these genetic variations with symptoms of IBS and high somatic symptom scores.

Methods: Validated bowel and somatic symptom questionnaires characterised the phenotype: 90 with IBS constipation (IBS-C), 128 IBS diarrhoea, 38 IBS alternating bowel function, and 20 chronic abdominal pain. Logistic regression analyses assessed associations of different polymorphisms for α2 adrenoceptor and SLC6A4 with IBS or chronic abdominal pain phenotypes and high somatic score.

Results: Two distinct polymorphisms independently appeared to be associated with the phenotype IBS-C: α2C Del 322–325 (odds ratio (OR) 2.48 (95% confidence interval (CI) 0.98, 6.28); p = 0.05) and α2A–1291 (C→G) (OR 1.66 (95% CI 0.94, 2.92); p = 0.08) relative to wild-type. Overall, the α2C Del 322–325 polymorphism (alone or combined with other polymorphisms) was also significantly associated with a high somatic symptom score (OR 2.2 (95% CI 1.06, 4.64); p = 0.03). Combinations of polymorphisms were also associated with high somatic scores.

Conclusion: Functionally distinct α2A and α2C adrenoceptor and serotonin transporter polymorphisms are associated with constipation and high somatic symptoms in patients with lower functional gastrointestinal disorders, although the strength of the genetic contribution to the phenotype is unclear.

IRRITABLE BOWEL SYNDROME

Irritable bowel syndrome (IBS) is a biopsychosocial disorder affecting 9–17% of patients of all ethnic groups. It is associated with abnormal gastrointestinal motor function, visceral sensitivity, and psychosocial or autonomic dysfunction. Psychosocial factors, particularly stress, can alter colonic motility, enhance colonic sensation, and influence the timing of patients’ presentation to physicians.

There is evidence of sympathetic adrenergic dysfunction in a subgroup of patients with IBS. Adrenergic agents alter the motor and sensory function of the human gastrointestinal tract. The most prominent effects in the human colon were observed with α2 agents. Twin studies suggest a genetic component in IBS. However, the influence of genetic factors that may modulate adrenergic and serotonergic functions in IBS is largely unknown.

Three human α2 adrenoceptor subtypes have been cloned and characterised: 2α, 2β, and 2γ subtypes. Prefunctional α2A and α2C adrenoceptor subtypes regulate the release of norepinephrine from sympathetic nerves through negative feedback at presynaptic nerve endings. The potential of the α2 adrenoceptor subtypes in the motor and sensory dysfunctions of disturbances of gastrointestinal function is shown in the model in fig 1. It is conceivable that polymorphisms of the genes encoding for these receptors may result in loss of normal synaptic autoinhibitory feedback and enhanced presynaptic release of norepinephrine. Synaptic levels of norepinephrine are modified by the norepinephrine transporter (NET); a mutation of NET in one family has been associated with autonomic dysfunction. Genetic disorders of α2 mechanisms could conceivably alter functions of relevance to IBS: gut motility, pain sensation, autonomic imbalance, anxiety, and somatisation.

Serotonin (5-hydroxytryptamine (5-HT)) modulates sensorimotor functions in the digestive tract. There are seven subclasses of serotonergic receptors, differentiated on the basis of structure, molecular mechanism, and function. The actions of enteric 5-HT are terminated by reuptake by the serotonin transporter (SERT). SERT in the gut is similar to that in the brain of the same species; gastrointestinal motility is abnormal in SERT knockout mice. The approved gene symbol for SERT is SLC6A4 (solute carrier family 6 (neurotransmitter transporter, serotonin), member 4); this abbreviation will be used in the remainder of this manuscript. Adaptive changes occur in the subunit composition of enteric 5-HT3 receptors in SLC6A4 knockout mice. Such changes are reflected in altered 5-HT3 receptor affinity and desensitisation and hence in function in response of the...
receptor polymorphisms. 2A and 2C adrenoreceptor polymorphisms. \( \alpha_{2A} - 1291 \) (C→G), cytosine to guanine transversion in promoter of \( \alpha_{2A} \) receptor protein; \( \alpha_{2A} \)Lys251, a cytosine to guanine transversion at position 753 that changes amino acid 251 of the third intracellular loop of the \( \alpha_{2A} \) adrenoceptor from asparagine to lysine; \( \alpha_{2C} \) Del 322–325, deletion in third intracellular loop of \( \alpha_{2C} \) adrenoceptor protein. Middle: norepinephrine transporter (NET). Bottom: solute carrier family 6 (neurotransmitter transporter, serotonin, member 4 (SLC6A4) (serotonin transporter promoter (SERT-P)).

There is also evidence that the two biogenic amines, serotonin and norepinephrine, interact in modulating gastrointestinal functions. For example, norepinephrine causes 5-HT release from enterochromaffin cells in mouse ileal tissues via \( \alpha_{2} \) adrenoceptor subtypes coupled to a pertussis toxin sensitive G protein\(^{21} \) or via \( \beta_{2} \) adrenoceptors in rat duodenal mucosa.\(^{22} \) Thus we were interested in exploring the potential combined effects of genetic mechanisms that influence serotonin and norepinephrine. Similarly, under-activation of serotoninergic function and overactivation of noradrenergic function modulate the brain circuitry involved in euthymic and abnormal mood and anxiety states.\(^{23} \) Thus altered control of noradrenergic and serotoninergic systems may result in symptoms of depression and anxiety, which are frequently associated with IBS at the time of presentation to physicians.

Selection of candidate genes for association studies

\( \alpha_{2A} \) and \( \alpha_{2C} \) adrenoceptor polymorphisms can act synergistically to alter the feedback regulation of norepinephrine release through their effect on the prejunctional \( \alpha_{2} \) adrenoceptor.\(^{24} \) Moreover, most of the presynaptic receptors inhibiting acetylcholine release are of the \( \alpha_{2A} \) subtype.\(^{25} \) \( \alpha_{2A} \) Adrenoceptors are located on somatic and visceral afferents and may be associated with reporting of chronic abdominal pain and somatic symptoms.\(^{26} \) \( \alpha_{2B} \) Adrenoceptor gene polymorphisms were not included in our candidate gene approach as the biological action of \( \alpha_{2B} \) adrenoceptors on vascular function (for example, hypertension) appears dependent on a sodium retention state, and renal medullary actions (for example, release of nitric oxide) also counteract the hypertensive effect of norepinephrine\(^{27, 28} \) mediated through the \( \alpha_{2B} \) adrenoceptor.

Norepinephrine reuptake requires a transporter protein (NET). A mutation of the gene encoding NET\(^{29} \) results in a non-functional NET, increased norepinephrine, and functional overstimulation of the sympathetic nervous system in response to physiological stimuli, such as orthostatic hypotension syndrome, neurocirculatory asthenia, and chronic fatigue syndrome.

The actions of enteric 5-HT are terminated by 5-HT transporter mediated uptake. We elected to study a polymorphism in the functional promoter region. This has been associated with gastrointestinal and neurobiological dysfunctions,\(^{30} \) neuropsychiatric disorders,\(^{31} \) and with altered response of IBS patients to a 5-HT\(_{3}\) antagonist.\(^{32} \)

Homoygous wild-type or long alleles reflect normal function. For the association studies performed, we assumed that the gene confers a functional disadvantage if there was a homozygous (short) or heterozygous polymorphism. This was based on the literature that shows that the heterozygote state in knockout mice confers a change in biological function that mirrors that of the homozygous state. Thus, for example, marked increases in the stress hormone adrenocorticotropin were found in the plasma of homoygous +/- and heterozygous +/- knockout mice compared with their control littermates. These data suggest that homoygous short and heterozygous SLC6A4 genotypes in mice are both associated with an increased stress-responsive phenotype.\(^{33} \) Moreover, the SLC6A4 heterozygous state was associated with reduced colonic transit response to alosetron compared with wild-type.\(^{34} \) There is also evidence that heterozygous variation in \( \beta_{2} \) receptors may impart a change in some \( \beta_{2} \) mediated functions or responses to medications. \( \beta_{2} \) Agonist treatment of mice heterozygous for the \( \alpha_{2A} \) adrenoceptor (\( \alpha_{2A} \) adrenoceptor +/-) lowers blood pressure without inducing sedation.\(^{35} \) Thus there is compelling evidence to hypothesise that the heterozygous polymorphisms may be biologically relevant.

**Study hypothesis and aims**

Our hypothesis is that single or combined polymorphisms of \( \alpha_{2} \) adrenoceptors and serotoninergic receptors are associated with IBS phenotypes and somatic symptom scores. To assess the potential role of genetic determinants in IBS, we considered five polymorphisms in four candidate genes or their promoters. The polymorphisms selected for study modify adrenergic or serotoninergic functions, and have been previously demonstrated to affect smooth muscle function, visceral sensation, bioamine metabolism, sympathetic function (for example, cardiac or vascular tone), or psychological state.\(^{36–38} \)

The aims of this study were: (1) to compare the distributions of polymorphisms of \( \alpha_{2A} \) and \( \alpha_{2C} \) adrenoceptors, norepinephrine transporter, and serotonin transporter protein promoter in patients with lower functional gastrointestinal disorders (FGID—that is, IBS or chronic functional abdominal pain (CAP)) and in healthy controls; and (2) to assess the association of these polymorphisms with phenotypes of IBS and CAP, and with high somatic symptom scores.

**METHODS**

**Asymptomatic healthy controls and patients with IBS**

All participants (18–75 years) completed a validated bowel disease questionnaire (including questions that corresponded to Rome II criteria\(^{39} \)) and a somatic symptom checklist.\(^{38} \) The latter has been used extensively in the literature to identify patients with a propensity to report somatic symptoms.\(^{38} \) The somatic symptom score was summarised as a mean of the frequency and severity scores over the 16 items, each recorded on a scale of 0–4.

Symptoms surveyed were: headache, backache, wheezing, trouble breathing, difficulty sleeping, fatigue (tiredness), depression (feeling sad or blue), general stiffness, palpitations, joint pains, eye pain associated with reading, dizziness, weakness, nervousness (or shakiness), hot or cold spells, and
high blood pressure. Subjects were classified as having a high somatic score when a subject’s mean score across the 16 domains was >0.75, which was the 90th percentile of mean scores in the healthy participants in this study. We have used these two questionnaires extensively in epidemiological studies (for example, in patients with diabetes).17

IBS participants were selected from an administrative database of 752 patients with IBS residing within a 150 mile radius of Rochester, Minnesota, USA, and were recruited by mailing. All IBS patients had already been evaluated by a staff gastroenterologist using clinically indicated tests, including endoscopy, biopsies, and tests of rectal evacuation. Healthy volunteers were recruited by public advertisement in Rochester, Minnesota. All participants gave informed consent for the study which was approved by the Mayo Foundation Institutional Review Board.

There is evidence of a significant likelihood of category transitions18 between constipation predominant IBS (IBS-C) and functional constipation (FC), and similar transitions in the categories of diarrhea predominant IBS (IBS-D) and functional diarrhea (FD). Hence we have grouped patients and functional diarrhoea (FD) into a second group (designated IBS-D). IBS-C and FC into one group (designated IBS-C) and those with IBS-D and FD into a second group (designated IBS-D).

DNA analysis by polymerase chain reaction amplification, identification, and sequencing

Venous blood drawn from a forearm vein was stored as de-identified samples. We isolated DNA from whole blood from 394 participants by the alkaline lysis method using the QIAamp DNA Blood Maxi Kit, (Qiagen Inc., Valencia, California, USA). Molecular assays were adapted from previously published papers to detect the candidate mutations or polymorphisms of interest.

We used the polymerase chain reaction (PCR) based fragment length assays to identify polymorphisms for the $\alpha_2C$ and $\alpha_2A$ adrenoceptor coding regions and in the $\alpha_2A$ adrenoceptor promoter. We confirmed polymorphisms by direct sequencing. Where there was no restriction site available, as for NET adrenoceptor promoter. We confirmed polymorphisms by direct sequencing alone.

Sequences used in these studies were obtained from GenBank/EBI Data Bank, and accession numbers are given below. Briefly, we performed PCR amplification using TaKaRa LA Taq with GC buffers (TaKaRa Shuzo Co., Ltd, Japan) in a total volume of 50 $\mu$l containing 750 ng template DNA, 0.4 $\mu$m primers, 2.5 units TaKaRa LA Taq, 20 mM DNTPs, and GC buffer 1 containing 2.5 mM MgCl2. After denaturing DNA samples at 94°C for one minute, we set up cycling conditions at 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for two minutes followed by an extension at 72°C for five minutes. The polymorphisms were amplified using a Perkin Elmer Gene Amp 9700 PCR thermal cycler.

$\alpha_2C$ and $\alpha_2A$ PCR products were digested for one hour and visualised by electrophoresis using 3% metaphor agarose (BioWhittaker Molecular Applications Inc., Rockland, Maine, USA). Polymorphisms were confirmed by direct sequencing performed at the Mayo Molecular Biology Core Facility using an ABI PRISM 377 DNA sequencer with XL Upgrade and 96 well Upgrade (Perkin-Elmer Applied Biosystems, Foster City, California, USA).

Detection of polymorphisms in the $\alpha_2$ adrenoceptor subtypes

(A) Within the promoter region for the $\alpha_2A$ adrenoceptor, a C to G transversion results in a MspI restriction fragment length polymorphism located at −1291 base pairs upstream of the origin of transcription.19 This polymorphism is designated by the abbreviation $\alpha_2A−1291\ (C\rightarrow G)$. Genotype GG has been detected as an apolymorphic band of 174 bp, and genotype CC as two polymorphic bands of 121 and 53 bp. In a Japanese control group, 36% had C allele and 64% G allele, with GG homozygosity in 16%, CG heterozygosity in 40%, and CC homozygosity in 44%.20 In contrast, a study of White Swedish men revealed allele frequencies of 23% allele C and 77% allele G, with no CC, 46% CG, and 54% GG genotypes.21 Data from European American healthy controls have not been reported previously. Altered expression of the gene causes loss of receptor function.

GenBank accession number was #M23533. A 523 bp region containing the MspI polymorphic site was amplified by PCR using the following primers: sense position 661:

### Table 1  
Patient characteristics, prevalence of SLC6A4, $\alpha_2C$ Del 322–325, and $\alpha_2A−1291\ (C\rightarrow G)$ polymorphisms, and mean somatic score (SoSC) in 276 patients with lower functional gastrointestinal disorders (FGID) and 120 healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Literature controls* (%)</th>
<th>All Lower FGID Patients</th>
<th>IBS-C</th>
<th>IBS-D</th>
<th>IBS-Alt</th>
<th>CAP</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>120</td>
<td>276</td>
<td>90</td>
<td>128</td>
<td>38</td>
<td>20</td>
<td></td>
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<tr>
<td>Age (mean, range)</td>
<td>36 (18–72)</td>
<td>49 (18–82)</td>
<td>52 (23–82)</td>
<td>47 (18–77)</td>
<td>47 (18–69)</td>
<td>47 (26–78)</td>
<td></td>
</tr>
<tr>
<td>Sex (n (% female))</td>
<td>95 (79)</td>
<td>226 (82)</td>
<td>83 (92)</td>
<td>98 (77)</td>
<td>30 (79)</td>
<td>15 (75)</td>
<td></td>
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<tr>
<td>SoSC (mean (SD))</td>
<td>0.31 (0.31)</td>
<td>0.9 (0.6)</td>
<td>0.89 (0.57)</td>
<td>0.83 (0.55)</td>
<td>0.93 (0.65)</td>
<td>0.84 (0.62)</td>
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<td>Wild-type (%)</td>
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<td>40</td>
<td>32</td>
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<td>Heterozygous (%)</td>
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<td>Any polymorphism (%)</td>
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<td>64</td>
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<td>65</td>
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<td>$\alpha_2C$ Del 322–325</td>
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<td>Wild-type (%)</td>
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<td>85</td>
<td>91</td>
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<td>Homozygous polymorphism (%)</td>
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<td>4</td>
<td>2</td>
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<td>Any polymorphism (%)</td>
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<td>6</td>
<td>11</td>
<td>15</td>
<td>9</td>
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<td>5</td>
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<tr>
<td>$\alpha_2A−1291\ (C\rightarrow G)$</td>
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<td></td>
<td></td>
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<tr>
<td>Wild-type (%)</td>
<td>57</td>
<td>48</td>
<td>52</td>
<td>45</td>
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<tr>
<td>Heterozygous (%)</td>
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<tr>
<td>Any polymorphism (%)</td>
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<td>51</td>
<td>48</td>
<td>55</td>
<td>45</td>
<td>47</td>
<td>45</td>
</tr>
</tbody>
</table>

SoSC, mean somatic symptom score (mean and often mean bothersome scores for 16 symptom “items”). Each “item” is on a scale of 0–4. Lower FGID group comprises the four groups: irritable bowel syndrome with predominant constipation (IBS-C), irritable bowel syndrome with predominant diarrhea (IBS-D), irritable bowel syndrome with alternating bowel function (IBS-Alt), and chronic abdominal pain (CAP).

*Genotype frequencies for controls are from: Serretti and colleagues,22 Small and colleagues, and Tsai and colleagues.23
Detection of polymorphism in the serotonin transporter protein promoter

A polymorphism in the 5′ promoter region (5′-HTT-LPR) of SLC6A4 (42) consists of a repetitive sequence of 22 bp located 1 kb upstream of the SLC6A4 transcription start site. This polymorphism is biallelic in most populations. The allele with the smallest number of repeats, commonly called the short (S) allele, has lower transcriptional activity, leading to marked reductions in messenger RNA levels, 5-HT binding, and 5-HT uptake in both platelets and lymphoblasts compared with the long (L) allele. In European-Americans, the S and L alleles have generally been observed to occur at frequencies of approximately 0.43 and 0.57 with wild-type (LL) 32%, heterozygous 49%, and homozygous short 19%. Identification of the polymorphisms in the promoter for SLC6A4, the serotonin transporter protein, was by PCR based fragment length polymorphisms. GenBank accession number was #X76753. We synthesised oligonucleotide primers flanking the long polymorphic region corresponding to the nucleotide positions 1671 sense 5′ -GCCGCTCTGAAATGCCAGCAC 3′ and position 2219 antisense 5′ -GGAGAAGCTG-ACC-CCTGAAAACGTG 3′ to generate 572 bp PCR amplified fragments.

Data and statistical analysis

Logistic regression models were used to estimate the associations (odds ratios (OR)) for specific phenotypes of IBS and high somatic symptom scores with the different adrenergic and serotonergic polymorphisms.

A high somatic symptom score was defined based on the subject’s mean score greater than the 90th percentile of healthy participants. The odds ratios (95% confidence interval (CI)) for a specific phenotype were computed from the estimated logistic regression model coefficients (and their standard errors) examining individual polymorphisms or their combinations relative to the homozygous type. Race and sex were included as covariates in each of the logistic regression models. The statistical software used for all analyses was SAS.

After the study was completed, we estimated the effect size detectable given the number of subjects in different subgroups included in this study.

RESULTS

Lower FGID symptoms and high somatic symptom scores

The symptom phenotypes of patients with lower FGID were: 90 IBS-C, 128 IBS-D, 38 IBS alternating bowel function, and

Detection of norepinephrine transporter (NET) gene mutation

A guanine (G) to cytosine(C) missense mutation in nucleotide 237 of the coding region of NET is associated with Ala457Pro mutation. For examination of the NET gene, upstream and downstream primers were synthesised as follows: sense 5′ CCGGAAACTCTCACATTTG 3′ and antisense 5′ CGCTGAATTGAGGATGCTGG 3′. GenBank accession number was #J03853. A 723 bp region containing the polymorphic site was amplified by PCR using the following primers: sense position 547: 5′ -CCACCATCGTCGGCCGTGTGGCTCATCT 3′ and antisense position 1165: 5′ -TCACACGGAGTGTTACTTCCCTCGGAGTT 3′. The Sty1 restriction enzyme (New England Biolabs, Beverly, Massachusetts, USA) was used at 10 units per reaction.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Distributions (%) of irritable bowel syndrome and chronic abdominal pain phenotypes, and controls, in each genotype category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (%)</td>
</tr>
<tr>
<td>SLC6A4 wild-type</td>
<td>137</td>
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<tr>
<td>Heterozygous</td>
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<tr>
<td>Homozygous polymorphism</td>
<td>74</td>
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<tr>
<td>Any polymorphism</td>
<td>259</td>
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<tr>
<td>547-1291 (C→G) wild-type</td>
<td>348</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>31</td>
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<td>Homozygous polymorphism</td>
<td>8</td>
</tr>
<tr>
<td>Any polymorphism</td>
<td>39</td>
</tr>
<tr>
<td>SLC6A4 Del 322–322 wild-type</td>
<td>210</td>
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<tr>
<td>Heterozygous</td>
<td>160</td>
</tr>
<tr>
<td>Homozygous polymorphism</td>
<td>22</td>
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<tr>
<td>Any polymorphism</td>
<td>182</td>
</tr>
</tbody>
</table>

* A total of 396 (276 patients and 120 controls) had SLC6A4, 387 had 547-1291 (C→G) genotypes assayed.

IBS-C, irritable bowel syndrome with predominant constipation; IBS-D, irritable bowel syndrome with predominant diarrhea; IBS-Alt, irritable bowel syndrome with alternating bowel function; CAP, chronic abdominal pain.

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20 CAP. The demographics of patients in the different subgroups are shown in table 1. Female participants predominated in the patient (82%) and control (79%) groups. Caucasian patients were 89% of controls and 97% of patients; there were 6% Asian and 3% Hispanic healthy participants. Other racial groups were <1% in the two groups.

Lower FGID was associated with significantly higher somatic symptom scores relative to healthy controls (p<0.05).

**Prevalence of polymorphisms in lower FGID**

\[
\alpha_{2A} \text{Lys251 and NET polymorphisms were not identified in the first 100 patients and 20 controls, and were not measured in the remaining participants.}
\]

The distributions of polymorphisms for SLC6A4, \(\alpha_{2C} \text{ Del 322–325, and } \alpha_{2A} \text{ -1291 (C-G)}\) were not significantly different between patients with lower FGID compared with controls (table 1). The distribution of these polymorphisms in the controls of the present study was similar to the controls (table 1). The distribution of these polymorphisms in different between patients with lower FGID compared with the remaining participants.

**Association of polymorphisms with somatic symptoms in lower FGID**

The mean somatic symptom scores, percentage of high somatic scores, and the associations with different polymorphisms are shown in table 5. Overall, the \(\alpha_{2C} \text{ Del 322–325 polymorphism (alone or combined with other polymorphism) was significantly associated with a high somatic symptom score (OR = 2.2 (1.06, 4.64); p = 0.03). Combinations of polymorphisms were also associated with high somatic symptom scores: } \alpha_{2C} \text{ Del 322–325 with SLC6A4 (OR = 5.0 (1.11, 22.22); p = 0.04) and } \alpha_{2C} \text{ Del 322–325 with } \alpha_{2A} \text{ -1291 (C-G)} \text{ (OR = 11.1 (1.15, 108.1); p = 0.04) relative to respective wild-type genotype(s). The significant OR for combined } \alpha_{2C} \text{ Del 322–325 with } \alpha_{2A} \text{ -1291 (C-G)} \text{ polymorphisms had a wide confidence interval due to the small sample size.**}

**Statistical power to detect associations**

Table 6 summarises the “degree of association” between the presence/absence of specific polymorphisms in \(\alpha_{2C} \text{ Del 322–325, } \alpha_{2A} \text{ -1291 (C-G)}, \text{ and SLC6A4 genes versus specific symptom subgroups (including overall FGID). This assessment was based on a comparison of the observed proportion of patients in the group without a specific polymorphism (number of patients divided by the sum of the number of patients plus number of controls) and the proportion that could have been detected among those with the corresponding polymorphism. The data indicate that clinically meaningful associations of these three candidate genotypes could have been detected with at least 80% power—for example, a difference in prevalence of wild-type versus polymorphic genotypes of 12% for all lower FGID, and 19–20% for IBS-C and IBS-D.
adrenoceptors. Functional alteration of both serotonin and noradrenergic control by the concurrence of 2C Del 322–325 adrenoceptor and SLC6A4 polymorphisms may also influence gastrointestinal functions.

The 2C adrenoceptor polymorphisms tested alter the third intracellular loop (which binds to G proteins) or synthesis of the receptor; association of distinct 2C adrenoceptor genotypes with the IBS constipation phenotype supports a role for genetic predisposition in IBS in twin studies and epidemiological reports. These genetic variations may alter manifestations of the disorder. 2C Del 322–325 and 2A −1291 (C→G) polymorphisms result in reduced prejunctional 2C adrenoceptor function, and norepinephrine released from the prejunctional site is not effectively inactivated by reuptake and subsequent monoamine oxidation. Increased synaptic norepinephrine may inhibit cholinergic enteric motor neurones, reducing gastrointestinal motility (for example, constipation). Moreover, 2A mechanisms directly alter gut motor function.

Genetic variation in 2C adrenoceptor functions may influence visceral sensation and behaviour in IBS. 2C Adrenoceptors on primary visceral afferents facilitate the transmission of pain to the dorsal horn neurone in the spinal cord, and those in high density in locus coeruleus neurones influence the sedative effects of 2C agonists, which may relieve psychological or somatisation disorders in IBS. 2C Adrenoceptors are involved in control of behaviour and those on spinal interneurones modify descending inhibitory pathways from the brainstem that downregulate the dorsal horn neurones and peripheral sensation. Thus loss of normal 2C adrenoceptor function may reduce descending modulation, thereby increasing the “sensitivity” of the dorsal horn neurone. Higher somatic scores with the 2C adrenoceptor polymorphisms may reflect a somatisation disorder, or altered peripheral pathways or central sensation (for example, spinal pathway sensitivity or induction of affective disturbance). Loss of function 2C adrenoceptor function may also result in reduced feedback through presynaptic receptors, thereby allowing continued release of norepinephrine which results in inhibition of excitatory (cholinergic) neurones in the gut and hence colonic transit delay.

The allele frequencies and genotype distributions observed in the healthy controls in this study illustrate the importance of having ethnic controls in these studies. Thus distributions for the 2A −1291 (C→G) polymorphism is very different in Japanese, Swedish men, and the predominantly female European American populations in our study. On the other hand, distributions for 2C Del 322–325, 2A Lys251, and SLC6A4 were very similar to those for Caucasians in the published literature.

Table 5  Mean somatic scores (SoSc) and percentage of abnormal SoSc for each genotype combination in patients with lower functional gastrointestinal disorders and healthy controls

<table>
<thead>
<tr>
<th>N</th>
<th>SLC6A4</th>
<th>α2C Del 322–325</th>
<th>α2A −1291 (C→G)</th>
<th>SoSc score (mean) (SD)</th>
<th>% High score</th>
<th>OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>Wt</td>
<td>Wt</td>
<td>Wt</td>
<td>0.67 (0.57)</td>
<td>33%</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>51</td>
<td>Wt</td>
<td>Het/hom</td>
<td>Wt</td>
<td>0.74 (0.49)</td>
<td>41%</td>
<td>1.4 (0.6, 3.1)</td>
</tr>
<tr>
<td>9</td>
<td>Wt</td>
<td>Het/hom</td>
<td>Wt</td>
<td>0.77 (0.65)</td>
<td>44%</td>
<td>1.7 (0.7, 4.7)</td>
</tr>
<tr>
<td>6</td>
<td>Wt</td>
<td>Het/hom</td>
<td>Het/hom</td>
<td>0.94 (0.24)</td>
<td>83%</td>
<td>11.3 (11.1, 100.1)</td>
</tr>
<tr>
<td>108</td>
<td>Het/hom</td>
<td>Wt</td>
<td>Wt</td>
<td>0.59 (0.57)</td>
<td>30%</td>
<td>0.8 (0.4, 1.7)</td>
</tr>
<tr>
<td>90</td>
<td>Het/hom</td>
<td>Het/hom</td>
<td>Wt</td>
<td>0.76 (0.58)</td>
<td>40%</td>
<td>1.3 (0.6, 7.8)</td>
</tr>
<tr>
<td>10</td>
<td>Het/hom</td>
<td>Het/hom</td>
<td>Het/hom</td>
<td>1.23 (0.79)</td>
<td>70%</td>
<td>5.0 (1.1, 22.2)</td>
</tr>
<tr>
<td>9</td>
<td>Het/hom</td>
<td>Het/hom</td>
<td>Het/hom</td>
<td>0.53 (0.43)</td>
<td>22%</td>
<td>0.6 (0.1, 3.4)</td>
</tr>
</tbody>
</table>

*The high somatic symptom score refers to >0.75, the 90th percentile for healthy controls.

*Estimated odds ratio (95% confidence interval) from logistic regression model adjusting for race and sex.

Wt, wild-type; Het, Heterozygous; hom, homozygous polymorphism.
The association \( p = 0.04 \) of the combination of \( \alpha_2C \) Del 322–325 and SLC6A4 polymorphisms with IBS is consistent with evidence that modulation of \( \alpha_2 \) adrenoceptor function may alter the biological effects of serotonin.\(^{23}\) An increased availability of 5-HT at the synapse resulting from decreased NET expression or function may alter the biological effects of serotonin.\(^{43}\) An increased availability of serotonin at the synapse resulting from decreased NET expression or function may alter the biological effects of serotonin.\(^{43}\) The lack of a significant association observed between IBS phenotype and SLC6A4 polymorphisms does not negate a potential role of this genetic variation in responses to stress (as recently demonstrated in depression, see below) or to responses to serotoninergic medications.\(^{32,33}\) SLC6A4 polymorphisms have been associated with a variety of psychological disorders and their response to therapy.\(^{23,43}\)

Further studies are needed to assess the biological effects of the combined \( \alpha_2C \) and SLC6A4 polymorphisms. Specific hypotheses that are plausible and should be addressed in future studies are: firstly, that \( \alpha_2C \) Del 322–325 is associated with a greater prevalence and colonic transit delay in IBS patients without the deletion; and secondly, that there is greater severity and frequency of pain and lower thresholds for sensation of rectal or colonic distension in patients with functional lower gastrointestinal disorders with the combined \( \alpha_2C \) and SLC6A4 polymorphisms compared with patients without the genetic polymorphisms.

Our study followed the standards recommended for appraising genotype prevalence and gene-disease association\(^{32}\): analytical validity by DNA sequencing to confirm results by PCR and restriction; blinding of investigators with laboratory personnel assessing genotype and clinicians categorising the phenotype; confirmation that genotype frequencies conform to those reported in controls\(^{36,40,41}\) from the same source population; and presentation of genotype frequencies. There were no patients with the mutation tested for NET or for the \( \alpha_2A \mathrm{Lys}251 \) polymorphism in the first 100 patients and 20 controls. However, the investigated NET mutation had been identified in a specific family with orthostatic intolerance syndrome.\(^{17}\) This mutation was not confirmed in a recent study of 14 patients with orthostatic intolerance syndrome.\(^{17}\) Therefore, we cannot exclude the possibility that alternative genetic alterations in the NET gene may be relevant to disorders of gastrointestinal function.

We assessed the power to detect associations between phenotypes and candidate genes. We detected no NET mutation or \( \alpha_2A \mathrm{Lys}251 \) polymorphisms in 120 predominantly Caucasian patients and controls. Absence of any genetic variation in these two candidate genes suggests that they are unlikely to play significant roles in determining IBS or somatic symptom phenotype. However, given the low prevalence of the \( \alpha_2A \mathrm{Lys}251 \) polymorphism in African-Americans, further studies in African-Americans are required. Table 6 summarises the “degree of association” between the presence/absence of specific polymorphisms in \( \alpha_2C \) Del 322–325, \( \alpha_2A \mathrm{~Lys}251 \), and SLC6A4 genes or combinations versus specific symptom subgroups (including overall FGID). It indicates that clinically meaningful associations of these three candidate genotypes could have been detected with at least 80% power.

In summary, we have shown that two functionally distinct \( \alpha_2 \) adrenoceptor polymorphisms, alone or in combination with a SLC6A4 polymorphism, are associated with constipation and high somatic symptom scores in patients with lower functional gastrointestinal disorders. These data suggest that genetic factors may interact with environmental factors and contribute to the manifestations of IBS; however, the strength of the genetic contribution to the phenotype is unclear. A gene-by-environment interaction was recently demonstrated in depression as SLC6A4 polymorphism influences the effect of life event stress on depression.\(^{52}\) Similar gene-environment interaction studies are needed in IBS in view of the evidence that life event stress is associated with IBS.\(^{2,4}\) models of visceral hypersensitivity,\(^{53}\) and depression.\(^{54}\) Moreover, in view of the reduced global IBS symptoms and bowel dysfunction in IBS patients treated with an \( \alpha_2 \) agonist\(^{55}\)
and modulation of μ opioid responses to pain by genetic variation in activation of norepinephrine by catechol-o-methyl transferase, pharmcogenomic studies would be of significant interest and potential clinical relevance.

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**REFERENCES**


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