IRRITABLE BOWEL SYNDROME

Association between a functional polymorphism in the serotonin transporter gene and diarrhoea predominant irritable bowel syndrome in women


Background and aims: Serotonin (5-hydroxytryptamine, 5-HT) is an important factor in gut function, playing key roles in intestinal peristalsis and secretion, and in sensory signalling in the brain-gut axis. Removal from its sites of action is mediated by a specific protein called the serotonin reuptake transporter (SERT or 5-HTT). Polymorphisms in the promoter region of the SERT gene have effects on transcriptional activity, resulting in altered 5-HT reuptake efficiency. It has been speculated that such functional polymorphisms may underlie disturbance in gut function in individuals suffering with disorders such as irritable bowel syndrome (IBS). The aim of this study was to assess the potential association between SERT polymorphisms and the diarrhoea predominant IBS (dIBS) phenotype.

Methods: A total of 194 North American Caucasian female dIBS patients and 448 female Caucasian controls were subjected to genotyping.

Results: A strong genotypic association was observed between the SERT-P deletion/deletion genotype and the dIBS phenotype (p = 3.07 × 10⁻⁵; n = 194). None of the other polymorphisms analysed was significantly associated with the presence of disease.

Conclusions: Significant association was observed between dIBS and the SERT-P deletion/deletion genotype, suggesting that the serotonin transporter is a potential candidate gene for dIBS in women.

The essential role of serotonin (5-hydroxytryptamine, 5-HT) in gut function is well recognised, and becoming better understood (see Gershon for review). 5-HT is contained primarily in enterochromaffin (EC) cells in the epithelial lining of the gut, and to a lesser degree in enteric neurones of the submucosal and myenteric plexuses. Multiple subtypes of receptor for 5-HT, located on terminals and cell bodies of these neurones (see Galligan for review), transduce visceral stimuli into motor and secretory activity. In addition, 5-HT receptors located on terminals of extrinsic spinal and vagal sensory nerves are involved in the signalling of events in the gut wall to the central nervous system (CNS). EC cells have been described as “secondary sense” cells, transducing luminal stimuli by releasing 5-HT from their basolateral membranes into the vicinity of afferent nerve terminals. This signalling mechanism is the primary method of sensory transmission from the lumen of the gastrointestinal tract (as no afferent neurone terminals penetrate the epithelial cell layer to directly sense luminal contents) and involves secretion of large amounts of 5-HT. For this reason, there is a need for an efficient mechanism for 5-HT reuptake, to prevent overstimulation/desensitisation of the sensorimotor responses, and indeed toxicity to other organs. Such reuptake of serotonin into mucosal epithelial cells and enteric neurones is mediated by a specific transporter protein known as the serotonin reuptake transporter (SERT), 5-HT transporter (5-HTT), or solute carrier family 6 member 4 (SLC6A4). This is the same molecule as that which performs reuptake in CNS neurones.

There are known pathophysiological consequences of excessive 5-HT release in the bowel, including diarrhoea (for example, cholera toxin induced1 12) and nausea and vomiting. Indeed, it has also been suggested that exaggerated release of 5-HT occurs in diarrhoea predominant irritable bowel syndrome (dIBS) patients following meals,14 and a trend for correlation of such exaggerated 5-HT release with postprandial symptoms of diarrhoea, abdominal discomfort, and pain, suffered by some IBS patients, has been reported.15 Furthermore, the therapeutic benefit of antagonists at 5-HT₃ receptors in female IBS patients with a diarrhoea predominant symptomology16 adds to the weight of evidence implicating dysfunction of the 5-HT system in this subtype of the disease. Increased plasma 5-HT levels could result from either exaggeration of its release or reduced reuptake, or a combination of both. Indeed, evidence exists for raised numbers of 5-HT containing EC cells in some dIBS patients.17 18 In addition, SERT dysfunction, or the recently reported19 reduction of SERT expression, could also result in

Abbreviations: 5-HT, 5-hydroxytryptamine; 5-HTT, 5-hydroxytryptamine transporter (= SERT); bp, base pair; CNS, central nervous system; dIBS, constipation predominant irritable bowel syndrome; del, deletion; dIBS, diarrhoea predominant irritable bowel syndrome; EC, enterochromaffin (cell); HWE, Hardy-Weinberg equilibrium; IBS, irritable bowel syndrome; ins, insertion; kb, kilo base pair; LD, linkage disequilibrium; PCR, polymerase chain reaction; SERT, serotonin reuptake transporter ( = 5-HTT); SLC6A4, solute carrier family 6 member 4; SNP, single nucleotide polymorphism; VNTR, variable number of tandem repeats

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raised 5-HT levels and contribute to symptoms in these patients. Elegant genetic manipulation studies in mice have investigated the latter possibility. The authors found that mice in which SERT was deleted demonstrated a gut function phenotype which was markedly different from wild-type littermates. The transgenic animals displayed altered bowel habit such that either diarrhoea (the majority) or constipation was observed. Interestingly, recent reports have described polymorphisms in the SERT gene which lead to either up- or downregulation of its expression. Such altered expression has thus far been reported in lymphocytes, platelets, and brain tissue, and although similar changes have not yet been investigated in the gut, it is possible that they would be found here also. The possibility exists that such polymorphisms may be present in subtypes of IBS patients, and the predicted resultant changes in 5-HT content (recently reported by Moses and colleagues) or function in these patients may underlie some of their symptomology and indeed response to drugs. To date, two studies have addressed these possibilities: Pata reported no association between SERT polymorphisms and the presence of IBS, regardless of subtype, but identified the short/short (s/s) homozygous polymorphism as a risk factor for a constipation predominant form of the disease (cIBS) and the long/short (l/s) heterozygous polymorphism similarly for dIBS. Camilleri and colleagues demonstrated greater inhibition of colonic transit in response to treatment with the 5-HT3 antagonist alosetron in dIBS patients with the SERT polymorphism genotype which produces increased expression of the transporter and thus presumably enhanced uptake of 5-HT. Although this study was not aimed at investigating disease association, another study from the same group, which has been reported in abstract form, suggested a trend towards significance for the odds ratio for the s/s polymorphism (versus the heterozygous genotype) to be associated with the presence of cIBS.

The SERT gene encoding the SERT protein is located on chromosome 17q11.2-q12. The main gene transcript of 2.5 kilo base pairs (kb) (Ensembl Accession No L05568) contains 14 exons which span approximately 31 kb of genomic DNA transcript. Some evidence of alternative transcript splicing has been reported in the literature. One of the first SERT polymorphisms characterised was the variable number of tandem repeats (VNTR) in intron 2 of the gene and consists of 17 base pair (bp) repeats. The SERT gene linked polymorphic region (SERT-P), originally known as 5HTT-LPR, was first reported in 1996 by Heils and colleagues. It consists of a 44 bp insertion/deletion in the 5’ flanking promoter region of the gene creating long (l) and short (s) allelic variants, respectively. The polymorphism is located within a GC rich region comprised of 20–23 bp repeat units with the s and l alleles having 14 and 16 repeats, respectively. The same study also reported evidence for allele dependent differential SERT promoter activity. Additional work has shown that cells homozygous for the insertion allele (l/l) exhibit a higher uptake of serotonin compared with those having one or two copies of the deletion allele (l/s or s/s). Therefore, this suggests that the presence of one or more copies of the S allele reduces not only the level of transcription but also levels of protein expression and reuptake of serotonin. Both the VNTR and SERT-P polymorphisms have been extensively investigated in genetic association studies for various complex behavioural traits and disorders such as bipolar disorder, obsessive-compulsive disorder, autism, schizophrenia, substance abuse, and eating disorders, and perhaps more interestingly with respect to the present study, in fibromyalgia, anxiety, and depression, which are thought to be comorbid with IBS.

<table>
<thead>
<tr>
<th>Sample set</th>
<th>Female Caucasian samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBS patients</td>
<td>194</td>
</tr>
<tr>
<td>North-American phase III clinical trials (diarrhoea predominant)</td>
<td>48</td>
</tr>
<tr>
<td>DNA Sciences Laboratories</td>
<td>125</td>
</tr>
<tr>
<td>Coriell</td>
<td>275</td>
</tr>
<tr>
<td>GlaxoSmithKline samples</td>
<td>418</td>
</tr>
<tr>
<td>Total</td>
<td>448</td>
</tr>
</tbody>
</table>

**Table 1** Samples genotyped for polymorphisms in the SERT gene

**MATERIALS AND METHODS**

**Patient and control populations**

A total of 194 samples from North American Caucasian IBS patients were used in this study. These patients were a subset of subjects who participated in the GlaxoWellcome phase III clinical trials (S3BA3001 and S3BA3002) for the alosetron hydrochloride compound (Lotronex) and were willing to participate in the genetic substudy after providing fully informed consent. All patients were females (age range 18–83 years) with a history of IBS (diarrhoea as the major bowel habit). These subjects had at least six months of recurrent symptoms that fulfilled the Rome I criteria for IBS, had normal sigmoidoscopic/colonoscopic results within five years and who, during a two week baseline period, had daily mild to severe abdominal pain/discomfort, and an average stool frequency of greater than 2.5 normal to watery stools/day. Patients were excluded if they had an unstable medical disorder, another gastrointestinal disorder, a major psychiatric disorder, or substance abuse within the previous two years. Further details of the study population and results of these two trials are published elsewhere.

No samples from patients with cIBS were collected in these clinical trials. A panel of 448 North American Caucasian random female control samples was collected from various sources (table 1). As the IBS status for these samples was largely unknown, the number of control samples collected was higher than the patient collection, allowing for an incidence of IBS of up to 20% in the general population. As the population was not selected on the basis of their IBS status, age of onset matching was not considered necessary.

**Single nucleotide polymorphism (SNP) identification**

All available genomic sequence (both exonic and intronic) surrounding the SERT exons (including 10 kb 5’ and 3’ flanking sequence) was amplified by polymerase chain reaction (PCR) in overlapping fragments on 30 random female Caucasian DNA samples (healthy volunteers with full consent) followed by direct sequencing of the product. The resulting sequence for these samples was aligned to allow for the detection of polymorphisms in each amplified fragment of the gene. Approximately 18 kb of genomic sequence was screened in total and a total of 17 SNPs were identified. Seven of these were selected for genotyping on the
The standard reaction mixture contained 8 ng of genomic DNA for the 5HTT-G160A assay where the reaction volume was 25 μl and primers used can be found in Table 3. All assays were performed in an 8 μl reaction volume containing 50 ng of genomic DNA, except for the 5HTT-G769T and G160A. All assays were processed using the following standard cycling conditions: 50 °C for two minutes, 95 °C for 10 minutes, 40 cycles of 95 °C for 15 seconds plus 62 °C for 60 seconds. When cycling was finished, reactions were kept at 15 °C until plates were finished. The 17 bp VNTR in intron 2 was analysed by PCR using the primers detailed in table 3. Amplification was performed in a 25 μl reaction volume containing 50 ng of genomic DNA.

### Table 2 Polymorphisms in the SERT gene genotyped on the diarrhoea predominant irritable bowel syndrome case/control samples

<table>
<thead>
<tr>
<th>Single nucleotide polymorphisms</th>
<th>GenBank Acc No</th>
<th>Sequence position</th>
<th>Location</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Minor allele frequency*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HTT-T623C</td>
<td>X76753</td>
<td>bp 623</td>
<td>5’ end genomic</td>
<td>T</td>
<td>C</td>
<td>(3.1%)</td>
</tr>
<tr>
<td>5HTT-T3287C</td>
<td>X76753</td>
<td>bp 3287</td>
<td>5’UTR</td>
<td>T</td>
<td>C</td>
<td>(7.3%)</td>
</tr>
<tr>
<td>5HTT-G674A</td>
<td>U79746</td>
<td>bp 674</td>
<td>Intron 1a</td>
<td>G</td>
<td>A</td>
<td>(9.4%)</td>
</tr>
<tr>
<td>5HTT-C867T</td>
<td>U79746</td>
<td>bp 867</td>
<td>Intron 1a</td>
<td>C</td>
<td>T</td>
<td>(37.6%)</td>
</tr>
<tr>
<td>5HTT-A2631C</td>
<td>X76762</td>
<td>bp 2631</td>
<td>Exon 1b non-coding</td>
<td>A</td>
<td>C</td>
<td>(17.4%)</td>
</tr>
<tr>
<td>5HTT-G160A</td>
<td>X76758</td>
<td>bp 160</td>
<td>Intron 8</td>
<td>G</td>
<td>A</td>
<td>(45.8%)</td>
</tr>
<tr>
<td>5HTT-G769T</td>
<td>X76762</td>
<td>bp 769</td>
<td>3’ UTR</td>
<td>G</td>
<td>T</td>
<td>(48.6%)</td>
</tr>
</tbody>
</table>

### Table 3 Nucleotide sequences for assay primers and probes for polymorphisms in the SERT gene

<table>
<thead>
<tr>
<th>Single nucleotide polymorphisms</th>
<th>Forward primer sequence 5’–3’</th>
<th>Reverse primer sequence 5’–3’</th>
<th>VIC probe sequence 5’–3’</th>
<th>Fam probe sequence 5’–3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HTT-T623C</td>
<td>TGC CTC TTC CCC GGT AGT G</td>
<td>ATC GCC ACA AGC ACC TTG TC</td>
<td>GCC CCG CCA TCG GAG GT</td>
<td>GCC CCA ACT TCA TCA TCA</td>
</tr>
<tr>
<td>5HTT-T3287C</td>
<td>CAC CCC GGC CCG TAG</td>
<td>AGA GGA TAG AAA ATG TGT GCA GGT A</td>
<td>CCA TCG CCT GAG GC</td>
<td>CCC TCC CTG GCG ACC GC</td>
</tr>
<tr>
<td>5HTT-G674A</td>
<td>GAA TTC AGG ACT GGA TGC CIT C</td>
<td>AAT GTG AAG AAC ACA CCT TGT CTG TCT</td>
<td>GAG AAC ACT TCG GAG</td>
<td>TGG ACC TCG TGG TTC</td>
</tr>
<tr>
<td>5HTT-C867T</td>
<td>CAC GGT A</td>
<td>GCA GGT A</td>
<td>GAC CCT CTC TGT CTC</td>
<td>TGG ACC TCG TGG TGG</td>
</tr>
<tr>
<td>5HTT-A2631C</td>
<td>CTG CTT CCC CCG TTA GCT CTT</td>
<td>GCA GGT AAG AAC ACA CCT TGT CTG TCT</td>
<td>CTC TCG CTC TCT CTC</td>
<td>TGG ACC TCG TGG TGG</td>
</tr>
<tr>
<td>5HTT-G160A</td>
<td>TGC TTT AAG GAA AAA AAC TGG</td>
<td>GTC CTA CAA ACA AGA TGT</td>
<td>CAT TCC TCC TCT GTA</td>
<td>TGG ACC TCG TGG TGG</td>
</tr>
<tr>
<td>5HTT-G769T</td>
<td>TTG AAG GCA CAG GGA TGC TA</td>
<td>CAG CAG CAC ATG GAT</td>
<td>GAG ACC CAG GAA</td>
<td>TGC TTA TCG TGG TGG</td>
</tr>
</tbody>
</table>

### Other polymorphisms

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Assay type</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HTT-VNTR</td>
<td>PCR</td>
<td>GTC AGT ATC ACA GGC TGG CAG</td>
<td>GTC AGT ATC ACA GGC TGG CAG</td>
</tr>
<tr>
<td>SERT-P</td>
<td>PCR</td>
<td>GAG GGA CTG ACG TGG ACA ACC AC</td>
<td>GAG GGA CTG ACG TGG ACA ACC AC</td>
</tr>
</tbody>
</table>

5-HTT, 5-hydroxytryptamine transporter (=SERT); PCR, polymerase chain reaction; VNTR, variable number of tandem repeats.
1 x Perkin-Elmer PCR buffer (no MgCl₂), 1.5 mmol/l MgCl₂, 0.2 mmol/l deoxynucleotide triphosphates, 10 pmol of each primer, and 1 unit of AmpliTaq Polymerase (Perkin-Elmer, Boston, Massachusetts, USA). DNA was denatured at 94 °C for four minutes and subjected to 35 cycles of 94 °C for one minute, 60 °C for one minute, and 72 °C for one minute, followed by a final extension at 72 °C for 10 minutes. Amplification products (10 µl) were resolved by electrophoresis on 4% NuSieve 3:1 agarose gels next to a 1 kb DNA molecular weight standard (Life Technologies, Carlsbad, California, USA) and visualised with ethidium bromide staining. The expected product sizes for alleles 9, 10, 11, and 12 are 253 bp, 270 bp, 287 bp, and 304 bp, respectively.

The 44 bp insertion/deletion polymorphism in the promoter region (SERT-P) was analysed by PCR using the primers detailed in table 3. Amplification was performed in a 25 µl reaction volume containing 200 ng of genomic DNA, 1 x Perkin-Elmer PCR buffer (no MgCl₂), 1.5 mmol/l MgCl₂, 0.2 mmol/l deoxycadenosine triphosphate, 0.2 mmol/l deoxycytidine triphosphate, 0.2 mmol/l deoxouridyidine triphosphate, 0.1 mmol/l deoxyguanosine triphosphate, 0.1 mmol/l 7-deaza- deoxyguanosine triphosphate, 20 pmol of each primer, 5% DMSO, and 0.5 unit of AmpliTaq Polymerase (Perkin-Elmer). DNA was denatured at 95 °C for four minutes and subjected to 35 cycles of 95 °C for 30 seconds, 57 °C for 30 seconds, and 72 °C for one minute followed by a final extension at 72 °C for seven minutes. Amplification products (25 µl) were resolved by electrophoresis on 3.5% NuSieve 3:1 agarose gels next to a 1 kb DNA molecular weight standard (Life Technologies) and visualised with ethidium bromide staining. The expected product sizes for the deletion (del or s) and insertion (ins or L) alleles are 484 bp and 528 bp, respectively.

**Statistical analysis**

**Testing for Hardy-Weinberg equilibrium (HWE)**

The genotype distribution for all SNPs, for both cases and controls, were tested for HWE using a χ² test (comment: the test was done for the number of expected genotypes, not the frequencies).

**Association test**

χ² tests were carried out to test for association between allele and phenotype (allelic association) and also between genotype and phenotype (genotypic association).

**Odds ratio**

Odds ratio and confidence intervals were estimated using SAS software. We applied the methods described in chapter 2 of Stokes and colleagues, specifically we used the “Proc Freq” procedure using the “Measures” option. Data for the del/ins and ins/ins genotypes were collapsed into one group to form a 2×2 table of “del/del” and “non del/del” individuals against disease status.

**Linkage disequilibrium (LD)**

LD is a measure of the association between alleles at different loci. LD tends to decline with distance between polymorphisms and generally exists for polymorphisms that are less than 100 kb apart. LD was measured using r² and D' statistics.

**RESULTS**

**Genotype analysis of SERT polymorphisms**

As the availability of SNPs in the public domain was limited when this study was performed, SNPs were isolated by resequencing all available genomic (exonic, intronic, untranslated region plus 10 kb flanking sequence) in 30 random female Caucasian DNA samples. A total of 17 SNPs were identified and a total of seven SNPs (available in the dbSNP database at http://www.ncbi.nlm.nih.gov/SNP/index.html; see table 2 for SNP reference ID) were selected for genotyping on the basis of their minor allele frequency (>5%). These seven SNPs were screened using the TaqMan 5' nuclease extension assay. PCR based assays were used to screen SERT-P and VNTR polymorphisms, which were performed in duplicate to control for genotyping error. A total of 194 IBS patient samples and 448 control samples were analysed for the nine polymorphisms (table 2).

**Association between IBS disease phenotype and SERT genotypes**

Statistical analyses were performed to investigate the association between genotypes and the dIBS phenotype.

**Genotype distribution for all polymorphisms was checked for deviation from HWE. Deviation was observed for the 5HTT-VNTR assay in the control study population (p = 0.0084). As this could suggest a problem with the assay, data related to this assay were excluded from further analysis.**

Association analyses were performed for the eight remaining polymorphisms. The strongest association was observed between the s/s genotype of the SERT-P polymorphism and the dIBS phenotype (p = 3.07×10⁻⁵). Table 4 shows the genotype distribution between dIBS cases and controls for this polymorphism. The percentage of s/s genotypes was nearly double in dIBS cases compared with controls at the expense of the heterozygote genotypes. The odds ratio for these data was 2.23 for s/s individuals versus non-s/s individuals with 95% confidence limits of 1.51–3.31, respectively, providing further statistical support for an association between the s/s genotype and the dIBS phenotype. Although deviation from HWE was also observed for the SERT-P polymorphism, this was seen for dIBS cases only (p = 3.1×10⁻⁶) and could be an effect of the genotypic disease association observed or the functional nature of this polymorphism. No association was observed with any of the other SERT polymorphisms.

LD analysis was performed to identify how many of the polymorphisms analysed in this study share the same block of LD (data not shown). The D' measurement of LD indicates that there may be breakdown of LD between the SERT-P and 5HTT-G674A polymorphisms. Also, none of the other polymorphisms are in perfect LD (r measurement >0.9) with the SERT-P polymorphisms. In fact, the only high r correlaton (>0.8) was observed between polymorphisms 5HTT-G160A and G769T.

Although association between the dIBS phenotype and a combination of two or more SERT polymorphism alleles (also called haplotypes) was also studied, none of the haplotypes showed a more significant association compared with the single polymorphism p values reported here (data not shown).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>dIBS cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>s/s</td>
<td>77 (17.2%)</td>
<td>61 (31.4%)</td>
</tr>
<tr>
<td>l/s</td>
<td>214 (47.8%)</td>
<td>62 (31.9%)</td>
</tr>
<tr>
<td>l/l</td>
<td>138 (30.8%)</td>
<td>63 (32.5%)</td>
</tr>
<tr>
<td>No genotype obtained</td>
<td>19 (4.2%)</td>
<td>8 (4.1%)</td>
</tr>
</tbody>
</table>

**Table 4** Genotype distribution and associated odds ratio for the SERT-P polymorphism in diarrhoea predominant irritable bowel syndrome (dIBS) cases and controls

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DISCUSSION

To date there has been little evidence for the involvement of genetic factors in the development of IBS. Although there have been indications from non-genetic studies that there may be familial associations in adults with IBS, including some twin studies, no candidate genes have been associated with any IBS phenotype. Recently, Pata et al. proposed the hypothesis that the SERT-P polymorphism in the serotonin transporter gene may be a risk factor for the disease, and this is supported by the findings of this study and others.

In our study, the distribution of the genotypes in the IBS cases clearly reflects an increase of the s/s genotype when compared with the control population. This is in stark contrast with earlier studies, the present data from a North American study population provide evidence of an association between the s/s genotype of the SERT-P polymorphism and IBS, which is perhaps somewhat more intuitively based on the predicted functional consequence of the polymorphism. The authors noted that these conclusions were drawn from very small samples and that interpretation of the results should be limited. However, in their pilot study involving 173 patients with IBS (mixed subgroup population) or chronic abdominal pain, Kim and colleagues also determined a trend towards an association between the s/s polymorphism and the constipated (IBS) phenotype. It is not immediately obvious, however, that a polymorphism that produces a reduced level of functional reuptake transporter, and elevated 5-HT levels, results in a constipated phenotype. It is not immediately obvious how a polymorphism with the reduced transporter expression and thus function, compared with the l/l variant, could lead to enhanced levels of 5-HT in the bowel of these patients. As described above, reduced transporter protein expression and less serotonin reuptake and thus function, compared with the l/l variant, could lead to enhanced levels of 5-HT in the bowel of these patients. Reduced transcription of the 5-HT transporter would be expected to result in a serotonin profile which is in high LD with the SERT-P polymorphism, may also be associated with the dIBS phenotype. The genotypic association is also reflected in the underlying pathophysiology of the disease, at least in part. In vitro findings in isolated cells and disease symptom expression is a huge leap and probably not a sensible link to try to claim.

Genotyping for the SERT-P polymorphism was performed in duplicate for all samples and the genotypes were scored in duplicate by different individuals, and thus it is unlikely that these results are due to genotyping error. This study has not determined how far the block of LD containing the SERT-P polymorphism extends upstream of the SERT gene and no LD map is available for the area surrounding the gene. Therefore, we cannot rule out the possibility that another polymorphism upstream of the SERT gene, which is in high LD with the SERT-P polymorphism, may also be associated with the dIBS phenotype. The genotypic association is also reflected in the HWE deviation observed for IBS cases, and this method of detecting disease association in a heterogeneous disease such as IBS has been reported in the literature.

There is no doubt that genetic studies on populations collected during clinical trials are unfortunately far from perfectly structured due to the strict limitation on the individuals that can be collected and their willingness to be involved in a genetic analysis. In the case of this IBS study, it was not possible to collect data from cIBS patients and so the analysis and conclusions are restricted solely to the dIBS population. Collection of matched controls was also problematic but this problem was addressed by increasing the number of controls used to over twice the number of cases. We have demonstrated (unpublished data) that when matched controls are not available, an increased number of controls from a general ethnic match increases study power and suppresses confounding effects such as population stratification and admixture. However, as the SERT-P polymorphism is known to show frequency variability between ethnic groups, additional studies based around the genetic hypothesis formulated in this work should be undertaken to replicate the association demonstrated.

It is interesting to speculate how the evidence of association between the s/s genotype of the SERT-P polymorphism with diarrhoea predominant IBS might provide some insight into the underlying pathophysiology of the disease, at least in this subgroup of female patients. As described above, reduced transcription of the 5-HT transporter would be expected to lead to enhanced levels of 5-HT in the bowel of these patients, which in turn might be predicted to result in increased bowel motility and secretion, and subsequent diarrhoea; a situation not dissimilar to that described for SERT knockout mice. Furthermore, as observed in the SERT−/− mice, it is intriguing to consider the possibility that such increased 5-HT may also lead to a constipated phenotype, as suggested by earlier studies. This may occur following 5-HT receptor desensitisation in the presence of high levels of agonist for long periods, or indeed could be produced by excessive stimulation of the 5-HT receptor bearing enteric neurones such that the normal motility pattern is stimulated to such an extent that coordination of enteric reflexes breaks down and contractile and secretory function fail. At a cellular level, such excessive neuronal depolarisation may result in “depolarisation block”. In this condition, the neuronal membrane
potential remains depolarised above the threshold for sodium channel activation, and further action potential generation is not possible. In this way, varying degrees of excitation of enteric neurones might contribute to both hyper- and hypomotility and secretion.

The potential effects of reduced uptake of 5-HT are not however restricted to the neurones of the enteric nervous system. Excessive or prolonged stimulation of extrinsic afferents may also result in the development of neuronal sensitisation, at peripheral, spinal, or higher CNS levels, such that perception of sensations from the bowel is heightened, resulting in symptoms of urgency, bloating, and pain. Such neuronal hypersensitivity is thought to be a key factor in the generation of sensory IBS symptoms, and can be mimicked in animals by repetitive physical stimulation of the bowel, or by periods of infection, or stress. In a similar way, repeated stimulation of either extrinsic afferent nerves by excessive 5-HT in patients with impaired reuptake may also provide another method by which sensory perception of events in the gastrointestinal tract may be inappropriately enhanced.

This study shows that the SERT polymorphism, or a polymorphism in linkage disequilibrium with the SERT polymorphism, may play a role in the development of IBS. We do not believe that the reduction in SERT is the sole cause of dIBS in our population but we do believe it might be a contributing factor. Perhaps a combination of this polymorphism with many other factors, such as raised EC cell number, could explain the variety of symptom patterns observed across the disease population. The recent finding of reduced SERT expression in IBS patient biopsies, coupled with our own findings, does encourage speculation that there is a role for altered transporter function in the disease. While research continues into the variety of possible factors which initiate, maintain, and exacerbate the symptoms suffered in IBS, the data reported in the present study clearly provide further support for the key role of the serotonin system in IBS pathophysiology and will hopefully stimulate further effort towards identifying and understanding further potential genetic and other factors that might contribute to the disease.

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Conflict of interest: All authors are or were employees of GlaxoSmithKline.

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