Supplementary materials

Methods

Exclusion criteria for both cohorts were: other GI disease(s) explaining the symptoms, severe disease(s) such as malignancy, heart disease, kidney disease or neurological disease, severe psychiatric disease or pregnancy.

Barostat testing

Subjects in both cohorts came after an overnight fast and received a rectal cleansing tap water enema (500-800 mL). A polyethylene balloon attached to a double-lumen polyvinyl tube (Salem Sump Tube, 18F; Sherwood Medical, Tullamore, Ireland) was inserted into the rectum, leaving the distal attachment site 5 cm from the anal verge. Distension to a maximal volume (650 mL) resulted in a spherical balloon shape. The catheter was connected to a computer-driven electronic barostat (Dual Drive Barostat, Distender Series II; G&J Electronics Inc, Toronto, Ontario, Canada). Two distensions at 25 mmHg each were performed *(Cohort1)*, or one distension sequence increasing in steps of 4 mmHg from 0 to 20 mmHg *(Cohort2)*, to unfold the balloon and familiarize the subjects with the barostat. The operating pressure (OP) was set to 2 mmHg above the minimal distending pressure (MDP) necessary to record respiratory variations in the balloon volume.

In *Cohort1*, an ascending method of limits (AML) rectal distension protocol [1] was performed *(Figure 1A)* with each phasic isobaric distension step (inflation speed 45mL/s) lasting 30 seconds and followed by 30 seconds at OP. Starting at OP, in every distension step the intra-balloon pressure was increased by 5 mmHg until pain was reported, or until a pressure of 70 mmHg was reached. During the last 10 seconds of each distension step, subjects were asked to rate their perceived rectal sensation as either no sensation, rectal fullness, urge to defecate, discomfort or pain. In this study we only used the pain threshold, defined as the distension pressure above OP at which the subject first reported pain. After the distension protocol, patients were asked to mark the location of their painful sensations on a schematic body map (scale 1:4) to assess the viscerosomatic referral area for pain, considered to reflect processing of sensory information at the level of the spinal cord [2].

In *Cohort2*, another AML rectal distension protocol[3] was used, with ramp inflation increasing with steps of 4 mmHg (inflation speed 45mL/s) without returning to OP between different steps, to identify thresholds for first sensation, desire to defecate, urgency, discomfort and pain *(Figure 1B)*. Starting at 0 mmHg, the distensions progressed with 4 mmHg increments every 60 seconds until pain was reported or until a pressure of 60 mmHg was reached. For this study, only the pain threshold is used in the analyses. After this AML protocol, the balloon pressure returned to OP before the second distension paradigm, where the subjects received 4 fixed phasic distensions at 12, 24, 36 and 48 mmHg above OP in random order, and were asked to complete VAS ratings for urge to defecate, gas, discomfort and pain after 30 seconds into each distension, but for this study only the pain ratings were used. The distensions lasted for 60 seconds with an inter-stimulus interval of 2 minutes with the balloon pressure at OP. The maximum pressure used for the random phasic distension was limited by the pain threshold from the previous AML; only one distension level above the AML pain threshold was delivered (e.g. if pain threshold in the AML was 30 mmHg above OP; distensions of 12, 24 and 36 mmHg above OP were delivered, but not 48 mmHg above OP). Few subjects completed all four distensions, therefore we choose to analyze the 36 mmHg distension with last value carried

forward if the 36 mmHg distension was not performed. Only pain ratings were used for the analyses in this study.

Questionnaire information

The Hospital Anxiety and Depression scale (HADS) is a self-report questionnaire consisting of 14 questions to assess severity of depression and anxiety symptoms using a 4-point Likert scale (0-3) [4]. The score is calculated for the 7 anxiety items and 7 depression items separately, resulting in 2 scores, one for anxiety (0-21) and one for depression (0-21), with high scores reflecting high symptom burden[5].

The Visceral Sensitivity Index (VSI) is a validated questionnaire to measure GI-specific anxiety[6] consisting of 15 statements covering 5 dimensions of GI-related cognitions and behaviors: worry, fear, vigilance, sensitivity and avoidance. Each question uses a 6-point response scale, and after conversion, the total scores range from 0 to 75, with a high VSI score indicating a high level of GI-specific anxiety.

The translated abuse questionnaire by Leserman and Drossman[7] was used to obtain information about four different abuse domains: childhood physical, childhood sexual, adult physical and adult sexual abuse. The subjects were categorized as having experienced abuse if any of the questions in that subcategory was answered 'yes' or, for the questions with a frequency rating, with a frequency of seldom or more often. The abuse questionnaire was added to the study about halfway through the recruitment of *Cohort 1* when a publication on the putative link between abuse and rectal sensitivity became available[8], so abuse data are only available for a subsample (n=124) of this cohort. All subjects in *Cohort 2* completed the questionnaire.

The Symptom Checklist-90-Revised (SCL-90R)[9] is a questionnaire developed to measure psychological symptom patterns of psychiatric and medical patients. This 90-item questionnaire uses a five-graded response scale (0-4), and consists of nine primary symptom dimensions and three global indices of distress. For the purpose of this study, only the dimension of somatization was used. The level of somatization is measured by the severity of somatic symptoms from different bodily systems with higher scores indicating more severe symptoms.

The PHQ-15 consists of 15 questions about the most frequent somatic symptoms from different bodily systems. The patients score the severity of each of the symptoms on a 3-point response scale (0-2), yielding a total score range between 0 and 30, with increased scores denoting increased somatic symptom severity (i.e. somatization)[10].

A limitation of both these self-report instruments is that they cannot distinguish between 'medically explained' and 'unexplained' symptoms [10], which is an important feature of the 'somatization' concept [11, 12]. In this study, adequate clinical and technical investigations were performed to rule out a medical explanation of GI and other potentially relevant symptoms, and major non-GI medical co-morbidity that may account for these somatic symptoms was ruled out by the physician on an "as needed" basis

Statistical methods

All analyses were performed in SAS version 9.4, and significance level was set to alpha=0.05. Results are presented as mean ± standard deviation (sd) unless otherwise stated.

The associations between measurements of visceral pain perception (*pain threshold, pain referral area, and pain intensity ratings during 36 mmHg phasic distension*) and questionnaire data (*sexual abuse in childhood, sexual abuse in adulthood, physical abuse in childhood, physical abuse in adulthood, anxiety, GI-specific anxiety, depression and somatization*) were tested using bivariate association analysis (correlations for continuous variables, independent samples Student's t-tests for dichotomous variables). Depending on the distribution of the variable, parametric or non-parametric testing was used as appropriate. The variables with significant associations (defined as $p<0.05$) in these bivariate analyses were included in general linear models (GLMs), controlling for age and gender in all models. As abuse date were only available in a subsample of *Cohort 1*, in case of a significant bivariate association between an abuse variable and one of the two dependent variables in this cohort, the corresponding GLM analysis was run on the subsample. In the GLMs the distribution of the residuals was taken into account. If normally distributed (*pain threshold Cohort 2*) regular GLM was used, if logarithmic transformation of the dependent variable resulted in normal distribution the log transformed values were used (*pain referral area Cohort 1*) and if not, the GLMs were performed on ranks (*pain threshold Cohort 1, pain intensity during rectal distension Cohort 2*).

The visceral sensitivity measurements were used as dependent variables in separate models. The independent variables were grouped according to: abuse (entered in the first step together with age and gender), anxiety and depression *(HADS anxiety, HADS depression, VSI)* and somatization *(standardized score on SCL-90R or PHQ-15)*. These three groups of independent variables were entered into the GLMs in three steps. By subsequently adding the variables we can evaluate independent effects as well as get a first indication of putative mediation effects. Several significant independent variables indicate that these factors are independently associated with the outcome variable (the visceral sensitivity measurement). If a variable changes from being significant to being not significant after adding a second variable that is significant in the new model, this suggests that the first variable may be mediated by the second variable, rather than having an independent/direct effect on the dependent variable.

Mediation in its strict sense implies a temporal order of the independent variable, mediator, and the dependent variable, which cannot be determined from this cross-sectional data set. The order in which the groups of variables were entered was therefore determined based on previous studies suggesting the following sequence of events: abuse → anxiety/depression → somatization → IBS (symptom severity) [13-15]. The temporal order of abuse preceding psychiatric symptoms/disorders has been shown in, among others, a longitudinal prospective study[16]. Other longitudinal studies have shown that anxiety and depression predict the development of IBS symptoms, but not the reverse[17], and that even though there is a bidirectional effect, anxiety and depression had a larger and more immediate effect on functional somatic symptoms (i.e. somatization) than the other way around[18]. In a general population sample without IBS symptoms, scoring high on several indicators of psychological distress was predictive of having IBS 15 months later[19]. These study results justify the order of entering abuse before anxiety and depression measurements, which in turn were entered before somatization.

In the instances where we found indication of mediation effects in the GLMs, the mediation effect was specifically tested using bootstrapping according to the method described by Hayes, using his purpose-built SAS macro INDIRECT[20] (http://www.afhayes.com/spss-sas-and-mplus-macros-and-code.html).

Discussion

Possible implications

There is strong evidence supporting that somatization is associated with chronic pain states and pain intensity ratings, and that somatization will improve with pain treatment[21], putatively by interfering with its underlying psychobiological central sensitization processes. Some drugs that have been tested in preclinical trials as treatments for central sensitivity (such as NMDA receptor antagonists, gabapentin, pregabalin and duloxetine) have been tested in IBS patients or in different human pain models [22-25]. Although hampered by small sample sizes and often lack of placebo control, some drugs targeting central sensitization[26] may reduce visceral sensitivity during rectal distension [23], block increased sensitivity to repeated noxious stimuli in patients with visceral and cutaneous hypersensitivity [27] and improve IBS symptoms including pain[22]. Based on our current findings, and the association between pain thresholds and IBS symptom severity [28], somatization questionnaires could possibly be used to identify IBS patients likely to benefit from these drugs, although prospective studies are obviously needed to confirm this hypothesis. Further, exposure-based cognitive behavioural therapy (CBT) has been shown to be effective in IBS, with its effects on GI symptoms being mediated by its effect on reducing GI-specific anxiety [29]. Based on these findings and our current finding of an association between GI symptom-specific anxiety and visceral sensitivity, this type of CBT could potentially decrease visceral sensitivity. Moreover, cognitively focused CBT has been shown to have a direct impact on GI symptoms rather than its effect being mediated by effects on psychological distress [30], but it remains to be elucidated whether these effects would be mediated by effects on somatization and/or visceral sensitivity. Finally, it has been shown that CBT is effective in reducing somatization outside the context of IBS [31], so we may speculate that it may be effective in reducing visceral sensitivity through these effects in IBS patients.

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