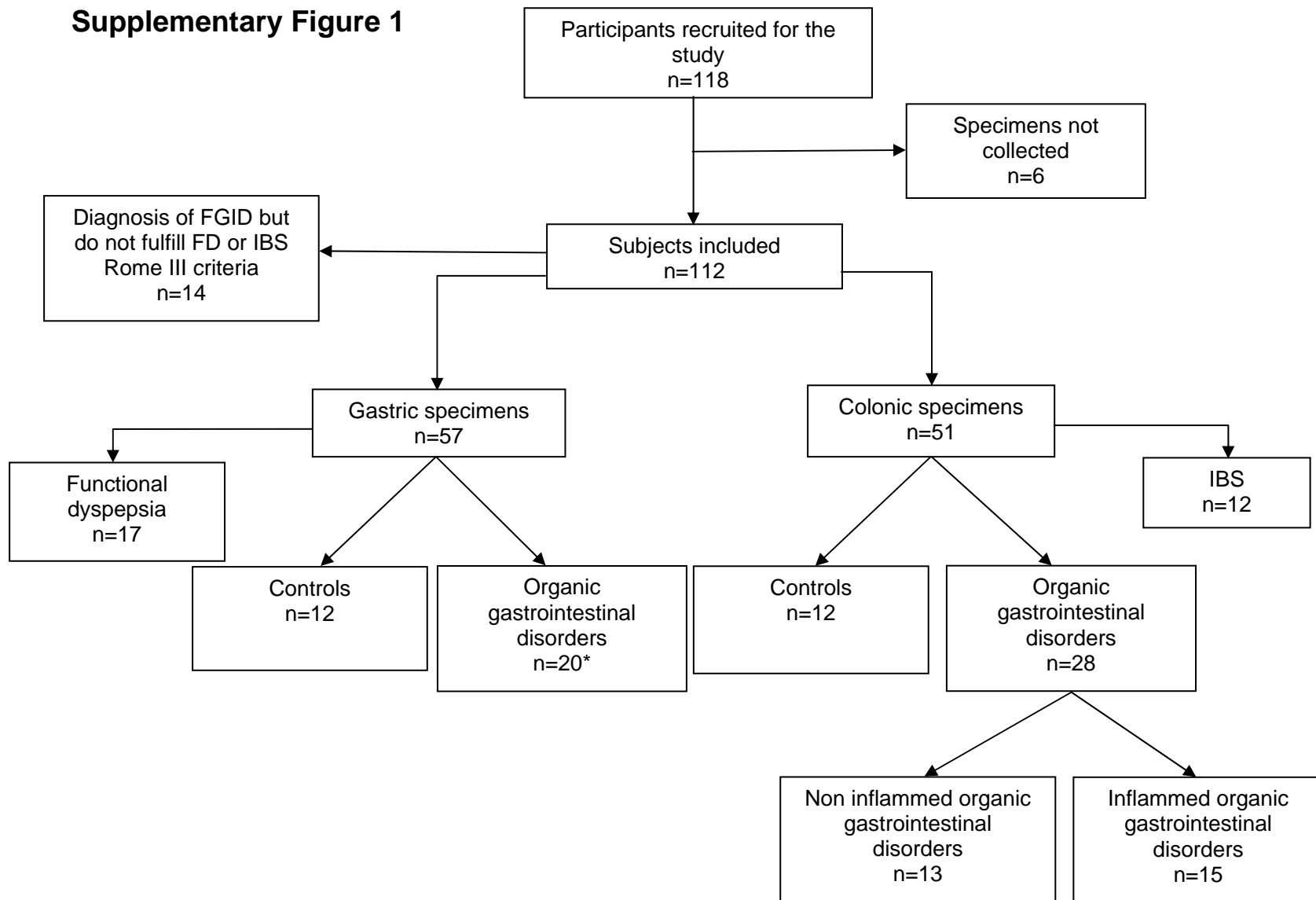
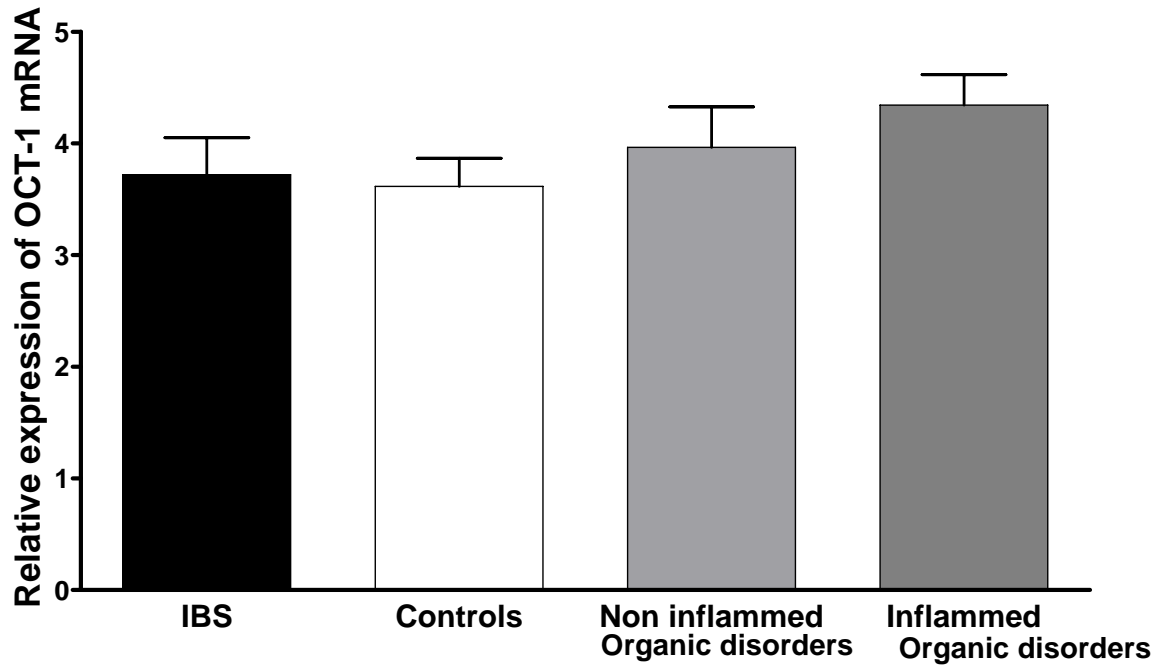


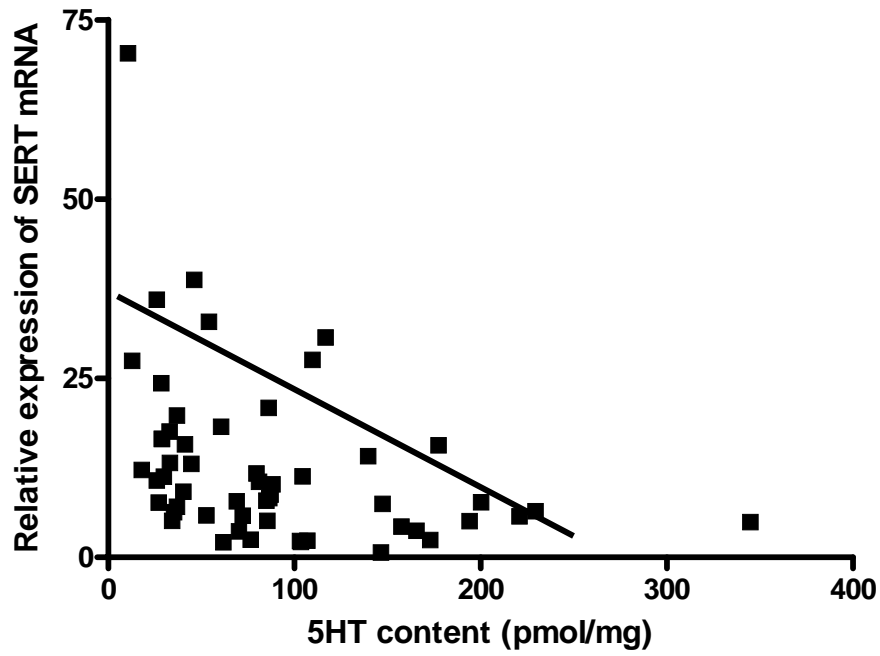
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Legends to supplementary Figures

Figure 1 (Supplementary Material) Distribution of the 118 children recruited for the study.

*The results obtained with the 8 gastric biopsies with inflammation grading ≥ 2 were not included in the analysis.

Figure 2 (Supplementary Material) Organic cation transporter-1 (OCT-1) mRNA relative expression in the rectal mucosa of patients with irritable bowel syndrome (IBS), control subjects and subjects with organic diseases with inflammation grading ≥ 2 (*Inflamed organic disorders*) and without inflammation (*Non-inflamed organic disorders*). No significant differences were detected in OCT-1 transcript levels of samples from patients with IBS as compared to controls and to subjects with *Inflamed organic disorders* and *Non-inflamed organic disorders*.

Figure 3 (Supplementary Material) Correlation between serotonin reuptake transporter (SERT) mRNA relative expression and serotonin (5-HT) content in the 51 colonic specimens. A significant, inverse correlation was found between SERT mRNA and 5-HT content ($r=-0.47$; $P=0.0003$).

Supplementary Table 1

Symptom severity variables in the study population with FGID

	IBS (n=12)	Functional Dyspepsia (n=17)
Pain frequency, Number (%)		
once a week	5 (42)	6 (35)
many times a week	3 (25)	6 (35)
everyday	4 (33)	5 (30)
Duration of pain, Number (%)		
2-3 months	6 (50)	2 (13)
4-11 months	2 (16)	12 (70)
≥ 1 year	4 (33)	3 (17)
Description of pain, Number (%)		
“A little”	1 (8)	2 (12)
From “a little” to “a lot”	9 (76)	10 (59)
“A lot”	2 (16)	5 (29)
Duration of pain episodes, Number (%)		
0-4 hours	8 (66)	10 (59)
Majority of the day	3 (25)	6 (35)
≥ 1 day	1 (8)	1 (6)
Missed days of school, Number (%)		
Never or < once / month	4 (33)	9 (53)
1-4 times / month	6 (50)	4 (23)
Many times a week	2 (17)	4 (23)
Missed social activities, Number (%)		
Never or < once / month	1 (8)	5 (29)
1-4 times / month	9 (76)	8 (47)
Many times a week	2 (16)	4 (23)

Supplementary Methods

Immunohistochemistry

One biopsy from each subject was fixed 2-3 hours in 2% paraformaldehyde / 0.2% picric acid and transferred in 70% ethanol at 4°C. Immunohistochemistry techniques were performed on an automate (NextES IHC, Ventana). Indirect immunoperoxidase staining was performed on the automate with the following primary antibodies: mouse anti-CD3 (Dako), mouse anti-human chromogranin A (Cedarlane Laboratories), mouse anti-human 5-HT (monoclonal H209, Dako). The first primary antibody was followed by incubation with the appropriate biotinylated secondary antibodies and with streptavidin and 3,3'-diaminobenzidine (DAB)-tetrachloride (ChemMate detection Kit, Dako). Slides were counterstained with hematoxylin. Lymphocytes were counted in the lamina propria respectively as CD3 positive cells within three to five non-overlapping high power fields at 40X magnification. Intraepithelial lymphocytes were enumerated as CD3 positive cells per 100 epithelial cells.

Numbers of enteroendocrine cells (chromogranin A immunoreactive) and EC cells (serotonin immunoreactive) were evaluated at the 40X magnification on the entire sections using a quantitative score (number of positive cells divided by number of glandular epithelial cells). All counts were performed blindly by an experienced pathologist (N.P.)

Measurement of RNA

One specimen from each individual was placed in a tube containing RNAlater™ (Qiagen). The tissue disruption was done using the MPBio Fast Prep using Lysing Matrix D (MP Biomedical) in 350 µL buffer RLT. Total RNA of one biopsy from the samples was extracted using the Qiagen Rneasy Mini kit with QIAshredders (Qiagen), and performed by the Qiacube (Qiagen). RNA was eluted in 30 µL water according to the manufacturer's instructions. RNA quantity and quality was determined for all samples using the NanoDrop (Thermo Scientific) and a BioRad Experion (BioRad). Reverse Transcription was performed using Promega reagents (Promega) according to manufacturer's instructions. For each RT reaction, 2 µg of total RNA was combined with 1 µl of oligo(dT) [500µg/ml] and enough water to bring the mix to 17 ml. This mix was heated for 10 min at 65°C and then put immediately on ice. For each sample 5 µl 5X RT buffer, 1 µl dNTP [10mM], 1µL Rnasin Ribonuclease Inhibitor [40u/µl], and 1µl M-MLV RT [200u/µl] was added. The sample were placed into a thermocycler and one cycle was run with the following profile, 42°C for 60min, 70°C for 15 min, with a 4°C hold.

The quantitative real-time polymerase chain reaction (qPCR) was performed with Quantifast™ SYBR® Green PCR and the QuantiTech Primer Assay (Qiagen) on an MxPro3000 instrument (Stratagen) according to the manufacturer's instructions. The master mix used for qPCR included: 12.5 µl of 2X Quantifast™ SYBR® Green PCR Master Mix, 2.5ml 10X QuantiTech Primer Assay, 7.5µl RNase-free water and 2.5 µl cDNA for genes tested or 9µl RNase-free water and 1 µl of cDNA for the references genes. The qPCR cycler conditions were 95°C for 5 min for initial activation step and 40

cycles were run with the following profile, 10 sec to 95°C, 30 sec for 60°C. The relative expression of serotonin transporter (SERT) (QT00058380), organic cation transporters (OCT1) (QT00019572) and tryptophan hydroxylase (Tph1) (QT00045346) in each sample were normalized by geometric mean of three reference genes (18S (QT00199367), Phosphomannomutase 1 (QT00013146) and hypoxanthine phosphoribosyl-transferase 1 (QT00059066)).²⁴ All primers were bought from Qiagen. The geometric mean of the three reference genes was similar in inflamed and non inflamed tissues as well as in the patients (IBS or FD) and controls.