Supplementary Data

The alternative serotonin transporter promoter P2 impacts gene function in females with irritable bowel syndrome

Sandra Mohr[1], Nikola Fritz[1], Christian Hammer[2,3], Cristina Martínez[1,4,5], Sabrina Berens[6], Stefanie Schmitteckert[1], Verena Wahl[1], Malin Schmidt[1,7], Lesley A. Houghton[8,9], Miriam Goebel-Stengel[10,11], Maria Kabisch[12], Dorothea Götze[1], Irina Milovač[13], Mauro D'Amato[14,15,16,17], Tenghao Zheng[14,15], Ralph Röth[1,18], Hubert Mönnikes[19], Felicitas Engel[6], Annika Gauss[20], Jonas Tesarz[6], Martin Raithel[21], Viola Andresen[22], Thomas Frieling[23], Jutta Keller[22], Christian Pehl[24], Christoph Stein-Thöringer[25], Gerard Clarke[26,27], Paul J. Kennedy[26,27], John F. Cryan[26,27,28], Timothy G. Dinan[26,27], Eamonn M. M. Quigley[27,29], Robin Spiller[30], Caroll Beltrán[31], Ana María Madrid[31], Verónica Torres[31], Wolfgang Herzog[6], Emeran A. Mayer[32], Gregory Sayuk[33], Maria Gazouli[34], George Karamanolis[35], Lejla Kapur-Pojskič[36], Mariona Bustamante[37,38], Raquel Rabionet[39], Xavier Estivil[37], André Franke[40], Wolfgang Lieb[41], Guy Boeckxstaens[42], Mira M. Wouters[42], Magnus Simrén[43], Gudrun A. Rappold[1,44], Maria Vicario[45], Javier Santos[45], Rainer Schaefert[46,47], Justo Lorenzo-Bermejo[12], Beate Niesler[1,18,44]

[1]Institute of Human Genetics, Department of Human Molecular Genetics, Heidelberg University Hospital, Heidelberg, Germany
[2]Department of Cancer Immunology, Genentech, South San Francisco, CA, USA
[3]Department of Human Genetics, Genentech, South San Francisco, CA, USA
[4]Institut de Recerca Biomèdica de Lleida (IRBLleida), Lleida, Spain
[5]Lleida Institute for Biomedical Research Dr. Pifarré Foundation (IRBLleida), Lleida, Spain
[6]Department of General Internal Medicine and Psychosomatics, Heidelberg University Hospital, Heidelberg, Germany
[7]Department of Translational Brain Research, Central Institute of Mental Health
[8]University of Leeds, St. James's University Hospital, Leeds, UK
[9]Mayo Clinic, Jacksonville, FL, USA
[10]Department of Psychosomatic Medicine, University Hospital Tübingen, Tübingen, Germany
[11]Department of Internal Medicine and Gastroenterology, HELIOS Clinic Rottweil, Rottweil, Germany
[12]Institute of Medical Biometry and Informatics, Heidelberg University, Heidelberg, Germany
[13]Faculty of Medicine, University Banja Luka, Banja Luka, Bosnia and Herzegovina
[14]School of Biological Sciences, Monash University, Clayton VIC, Australia
[15]Unit of Clinical Epidemiology, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden
[16]Department of Gastrointestinal and Liver Diseases, Biodonostia HRI, San Sebastián, Spain
[17]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain
[18]nCounter Core Facility, Department of Human Molecular Genetics, Heidelberg UniversityHospital, Heidelberg, Germany
[19]Martin-Luther-Hospital, Berlin, Germany
[20]Department of Gastroenterology, Infectious Diseases and Intoxications, Heidelberg University, Heidelberg, Germany
[21]University of Erlangen, Erlangen, Germany
[22]Israelitisches Krankenhaus, Hamburg, Germany
[23]Helios Klinik Krefeld, Krefeld, Germany
[24]Krankenhaus Vilsbiburg, Vilsbiburg, Germany,
[25]German Cancer Research Center, Heidelberg, Germany
[26]Department of Psychiatry and Neurobehavioral Science, University College Cork, Cork, Ireland
[27]APC Microbiome Ireland, University College Cork, Cork, Ireland
[28]Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland
[29]Lynda K. and David M. Underwood Center for Digestive Disorders, Houston Methodist Hospital, Weill Cornell

Medical College, Houston, TX, USA
[30]Nottingham Digestive Diseases Centre, University of Nottingham, Nottingham, UK
[31]Gastroenterology Unit, Hospital Clínico Universidad de Chile, Medicine Department, Universidad de Chile, Santiago de Chile, Chile
[32]Oppenheimer Center for Neurobiology of Stress, University of California, Los Angeles, CA, USA
[33]Washington University School of Medicine, St. Louis, MO, USA
[34]Laboratory of Biology, Medical School, National and Kapodistrian University of Athens, Athens Greece
[35]Academic Department of Gastroenterology, Medical School, National and Kapodistrian University of Athens, "Laikon" General Hospital, Athens, Greece
[36]University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina
[37]CRG, Centre for Genomic Regulation, Barcelona, Spain
[38]ISGlobal, Barcelona, Spain
[39]Department of Genetics, Microbiology and Statistics, Faculty of Biology, IBUB, Universitat de Barcelona; CIBERER, IRSJD, Barcelona, Spain
[40]Institute of Clinical Molecular Biology, Kiel, Germany
[41]Institute of Epidemiology, Kiel, Germany
[42]TARGID, University Hospital Leuven, Leuven, Belgium
[43]Institute of Medicine, University of Gothenburg, Gothenburg, Sweden
[44]Interdisciplinary Center for Neurosciences (IZN), Heidelberg University, Heidelberg, Germany
[45]Institut de Recerca Vall d'Hebron, Hospital Vall d'Hebron, Passeig de la Vall d'Hebron, Barcelona, Spain
[46]Department of Psychosomatic Medicine, Division of Internal Medicine, University Hospital Basel, Basel, Switzerland
[47]Faculty of Medicine, University of Basel, Basel, Switzerland

Formatat: espanyol (alfab. internacional)

Formatat: anglès (EUA)

SD Material and Methods

Tables

SD Table 1 Cohorts from IBS expert centers

IBS, irritable bowel syndrome; IBS-A, alternating IBS; IBS-C, constipation-predominant IBS; IBS-D, diarrhea-predominant IBS; IBS-M, mixed IBS; IBS-U, unspecified IBS; yrs, years.

Cohort		Female	Male	Age yrs (mean)	Rome II / III
	IBS-D	67	31	18-66 (41.6)	
	IBS-C	95	5	18-65 (40.1)	
UK1	Controls	60	32	18-63 (35.6)	II
	IBS-D	323	121	18-70 (44.3)	
	IBS-C	305	25	18-81 (43.7)	
"Belgium"= USA2&UK3	Controls	506	158	18-83 (41.1)	II
	IBS-D	12	2	24-77 (48.4)	
	IBS-C	15	1	22-80 (48)	
	IBS-M	31	9	19-58 (33.7)	
	IBS-U	1	0	54	
Chile	Controls	21	17	18-69 (32.7)	III
	IBS-D	72	42	19-79 (44.7)	
	IBS-C	40	8	16-71 (45.5)	
Germany1	Controls	138	203	n.a.	III
	IBS-D	70	41	13-79 (41.6)	
	IBS-C	18	7	18-76 (45.7)	
	IBS-M	75	47	14-88 (38.8)	
	IBS-U	7	2	27-74 (49.6)	
Germany2	Controls	510	651	19-77 (53.6)	III
	IBS-D	27	8	21-72 (44.2)	
	IBS-C	91	23	17-73 (51.7)	
	IBS-M	4	2	35-66 (56.2)	
Greece	Controls	74	69	19-94 (54.2)	III
	IBS-D	3	1	22-32 (27.25)	
	IBS-C	3	0	19-43 (20.3)	
	IBS-M	25	4	19-51 (29.6)	
	IBS-U	0	0	n.a.	
Ireland	Controls	38	12	18-50 (29.3)	III
	IBS-D	43	17	19-55 (36.9)	
	IBS-C	0	0	n.a.	
	IBS-M	0	0	n.a.	
	IBS-U	0	0	n.a.	
Spain	Controls	9	5	19-54 (25.7)	III
	IBS-D	16	17	19-60 (35.9)	
Sweden Mosaic	IBS-C	13	2	24-55 (32.3)	III

	IBS-M	58	22	18-60 (32.9)	
	IBS-U	1	0	37	
	Controls	25	9	19-53 (31.6)	
	IBS-D	47	17	20-71 (39.5)	
	IBS-C	26	9	19-61 (36.1)	
	IBS-M	10	3	21-53 (35.8)	
	IBS-U	31	9	21-64 (35.2)	
Sweden Sahlgrenska	Controls	43	18	20-68 (39.1)	II/III
	IBS-D	71	25	18-72 (45)	
	IBS-C	0	0	n.a.	
	IBS-M	0	0	n.a.	
	IBS-U	0	0	n.a.	
UK2*	Controls	30	84	19-67 (36)	III
	IBS-D	26	6	20-64 (40.1)	
	IBS-C	22	3	20-58 (39.6)	
	IBS-M	9	0	21-54 (33.2)	
	IBS-U	12	6	20-56 (33)	
	IBS-A	16	3	21-58 (40.8)	
USA1	Controls	59	34	19-58 (33.1)	II/III
	IBS-D	28	8	27-91 (54)	
	IBS-C	49	10	26-90 (54)	
	IBS-M	46	8	25-84 (55)	
USA3	Controls	48	46	17-80 (54)	III

SD Table 2 Gastrointestinal tissue samples used for expression analysis

[1]Spain (JS), [2]Germany (MR), [3]Germany (MGS).

Tissue samples				
GI Tissue	**IBS**	**Age (mean)**	**Controls**	**Age (mean)**
Jejunum[1]	23	22-51 (36)	26	19-53 (31)
Ileum[2]	16	23-82 (48)	11	36-72 (58)
Colon[3]	21	20-74 (48)	14	38-70 (59)
Sigma[2]	16	25-82 (52)	9	36-72 (57)

SD Table 3 Summary of genotyped samples from different countries

UK1 (Houghton), Germany1 (Mönnikes), Germany2* (IBS-Net Germany), UK2* (Spiller), UK3 (GLAXO), Ireland* (GC), USA1* (EAM), USA2 (GLAXO), USA3 (GS), Sweden* (MS), Spain* (JS), Greece* (MG), Chile* (CB). *GENIEUR
Additional controls: Germany 2 (PopGen), Sweden (SALT), Spain (INMA).
IBS, Irritable Bowel Syndrome; IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; IBS-M, IBS with mixed bowel habits; IBS-U, undetermined IBS.

Genotyped cohorts							
		IBS	IBS-C	IBS-D	IBS-M	Controls	Total
Discovery	UK1	197	99	98	0	90	287
	Germany1	169	50	119	0	475	644
	Germany2*	267	25	111	122	1161	1428
	UK2*	120	0	120	0	107	227
	UK3	190	83	107	0	149	339
	Ireland*	34	3	4	27	50	84
	USA1*	105	26	32	28	93	198
	USA2	455	185	269	0	487	942
	USA3	156	59	36	54	93	249
	Sweden*	196	30	53	87	2033	2229
	Spain*	60	0	60	0	1207	1267
	Greece*	155	114	35	6	143	298
Validation	Chile*	71	16	14	40	40	111
Pooled	Pooled	2175	690	1058	364	6128	8303
	Mean	167.31	53.08	81.38	28.00	471.38	638.69

SD Table 4 Primers for mutation analysis and genotyping
T_A, annealing temperature; bp, base pairs

Amplicon (Size)	Primer name	Sequence (5' - 3')	Length (bp)	TA (°C)	Method
SERT Promotor P2 (497bp)	SERT-P2-1for	CAACACAACCACTGACTACCAAG	23	59.6	Sequencing
	SERT-P2-1rev	CAGTCACTAGGGTTTGGCGT	20	60.2	
SERT Promotor P2 (450bp)	SERT-P2-2for	GGCAACAATCGATCCCTTTA	20	59.9	
	SERT-P2-2rev	CTCTGTTTCTTCTGGGCCTG	20	60.0	
SERT Promotor P2 (500bp)	SERT-P2-3for	AGAAGGGTCCAGTGTCATCTC	21	52.9	
	SERT-P2-3revb	CCTCCGGCTGTGTCCAGT	18	55.5	
SERT Promotor P2	SERT-P2rs2020938A1	GAAGGTGACCAAGTTCATGCTGCACTTGTTGGGC	40	87.6	KASPar® Assay
	SERT-P2rs2020938A2	GAAGG TCGGAGTCAACGGATTCGCACTTGTTGGC	41	89.1	
	SERT-P2rs2020938C1	TCAGAATTCATATCCTTCTGAGTGGTGG	29	68.1	
	SERT-P2rs2020938C2	ATATCCTTCTGAGTGGTGTTTGCATTCTT	29	68.8	

SD Table 5 Primers for expression analysis

T_A, annealing temperature; bp, base pairs

Amplicon (size)	Primer name	Sequence (5' - 3')	Length (bp)	TA (°C)
SERT Ex 1(a)/2 (507/600bp)	SERT ex1a for	CCAGCCCGGGACCAG	15	62.4
	SERT ex3 rev1	CCCCTCCATTCTGGTAACAT	20	58.7
SERT Ex 1(a/b)/2 (409bp)	SERT ex1c for	AAGGGCTCAAATTTCTAC	18	49.0
	SERT ex2 rev3	GGGTGGGAACAACCTTCTGT	20	60.0
ARF (336bp)	ARF-1-for	GCCAGTGTCCTTCCACCTGTC	21	65.0
	ARF-1-rev	GCCTCGTTCACACGCTCTCTG	21	65.0
qPCR Taqman Primer				
SERT Promoter P1 (211 bp)	SERT-P1_for	CACAGCCAGCGCCGC	15	72.0
	SERT-P1_probe	TGCCTCGAGGGCGCGAGG	18	77.0
	SERT-P1_rev	CGTCGGGATTGACACGTCG	19	69.0
SERT Promoter P2 (195 bp)	SERT-P2_for	GGCACAGGGGGACGGC	16	74.0
	SERT-P2_probe	CGGCGACACCAACCACCCCAG	21	77.0
	SERT-P2_rev	CGTCGGGATTGACACGTCG	19	69.0

SD Table 6 Target sequences for nCounter analysis

*P2 driven isoform; HK, housekeeping gene

Gene	Accession	Position	Target sequence	Type
ARF1	NM_001024227.1	1371-1470	CAATTCTGCATGGTC…..TGCTCCCACGGTTCC	HK
SNX17	NM_014748.2	1786-1885	CTTTCCTTGTCCCCT…..TATTTTGCACAAAGTC	HK
SDHA	NM_004168.2	2223-2322	AGTACATTGAAGGGA……CTTGCTTCATTCTTG	HK
HPRT1	NM_000194.1	241-340	TGTGATGAAGGAGAT…..TTACATCAAAGCACTG	HK
PPIA	NM_021130.2	926-1025	GGAATATTGAAAATG……AGGCAGAAGACCACCT	HK
PGK1	NM_000291.3	965-1064	ATTGTCAAAGACCTA……GATGAGAATGCCAAGA	HK
UBB	NM_018955.2	796-895	CACCTGGTCCTGCG…..TAGCCATTTGCCCCAA	HK
*SLC6A**	NM_001045.5	357-456	GTCTCCTGGAGGCA…..AAGAAGAACAAATGAGT	Target

SD Table 7 Primers for sequencing and cloning of the promoter P2 region
T_A, annealing temperature; bp, base pairs

Amplicon (Size)	Primer name	Sequence (5' - 3')	Length (bp)	TA (°C)
Cloning primers				
Promoter P2	SERT-P2Xhol-2	ATTACTCGAGTGGTGGGTGAGAGGATGTGTGGT	33	61.0
	SERT-P2-HindIII	ATTAAAGCTTCCTCCGAGCTCTCTATCGTCGGG	33	61.0
Gene specific primers				
SERT Promoter P2	SERT-P2-trev	CAGTCACTAGGGTTTGGCGT	20	60.2
	SERT-Ps-2for	GGCAACAATCGATCCCTTTA	20	59.9
	SERT-P2-2rev	CTCTGTTTCTTCTGGGCCTG	20	60.0
	SERT-P2-3for	AGAAGGGTCCAGTGTCATCTC	21	52.9
	SERT-KLON-13031-for	TAGCTGATGAGCTGGGGTGCCTC	23	72.5
	SERT-KLON 13161-for	GACTGTCCTGCCTGCTGAAAGGC	23	73.3
Vector Primers				
pGL3 Basic	pGL3 RVprimer3	CTAGCAAAATAGGCTGTCCCC	21	60.0
	pGL3 Seq Reverse	CAGGGCGTATCTCTTCATAGC	21	60.0

Patients

GlaxoSmithKline cohort

Two independent cohorts of IBS patients and controls from the UK (termed UK3) and the USA/Canada (termed USA2) from Glaxo, previously termed 'Belgium samples'[1,2] were included in this study. Informed consent was obtained from all participants and local ethics committees approved the study protocol. The demographics and clinical characteristics of both cohorts have been reported[3]. The IBS patients were divided into subgroups based on the predominant bowel habit according to the Rome II criteria. All patients were of Caucasian ethnicity[3].

Controls

PopGen Controls

Further German control data were taken from the PopGen Controls (https://www.epidemiologie.uni-kiel.de/node/119). The rs2020938 SNP was extracted from

data of 1,228 individuals. Thirteen SNPs not genotyped in PopGen were imputed into 1,228 PopGen individuals based on 2,577 reference individuals from phase 3 of the 1,000 Genomes Project. IMPUTEv2 was used to impute genotypes (default settings) based on resulting genotype probabilities, and exact genotypes were determined according to the 0.9 threshold rule (calls with uncertainty greater than 0.1 were treated as missing and the rest were treated as hard calls).

Spain: INMA (INfancia y Medio Ambiente)

Genotypes of further Spanish controls were taken from the INMA (INfancia y Medio Ambiente – Environment and Childhood) project. This is a network of birth cohorts in Spain that studies the effect of exposure to environmental pollutants in air, water, and diet during pregnancy on early childhood growth and development (http://www.proyectoinma.org/)[4]. The study had been approved by the ethical committee of each participating center and written consent was obtained from participating parents. Children from the following sub-cohorts participated in this study: INMA Menorca, INMA Sabadell, and INMA Valencia.

Sweden: SALT (Screening Across the Lifespan Twin) study

Additional genotypes of Swedish controls (from the SALT study) were selected from a published IBS GWAS study on a Swedish general population cohort[2], where 534 IBS patients defined based on Rome criteria and 4,932 asymptomatic controls were included in the analyses. In this study, healthy controls were selected based on reporting no bowel symptoms in an interview. We randomly chose 2,000 Swedish controls for replication studies.

Cell lines

Cell lines for expression and functional assays were obtained from the American Tissue Culture Cooperation (ATCC, LGC Standards, Wesel, Germany). These were Caco 2 (human epithelial colorectal adenocarcinoma), Colo 320 (human colorectal adenocarcinoma),

Formatat: espanyol (alfab. internacional)

HEK293T (human fetal renal cell carcinoma, which are highly transfectable as they carry the large T antigen of Simian virus 40) and SH-SY5Y as well as IMR-32 (both human neuroblastoma)- Cells were cultured as recommend by ATCC.

SD Methods

Sequencing of *SLC6A4* promoter region P2

Polymerase chain reaction (PCR)

PCRs were performed in 12.5 µl volumes containing 25–50 ng of genomic DNA as template, 3.125 pmol of each primer, 5 pmol of each dNTP (MBI Fermentas, St. Leon-Rot, Germany), 1.25 µl HotStarTaq PCR Buffer 10x concentrated (contains Tris-HCl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$), and 0.625 U (0.125 µl) of HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany). Thermal cycling was performed in Mastercycler *vapo.protect* thermal cyclers (Eppendorf, Hamburg, Germany). Annealing temperatures (T_A) and sequences of the promoter region P2 specific *SLC6A4* primers are shown in Supplementary SD Table 4. Cycling conditions were: initial denaturation at 95°C for 15 min followed by 35 cycles of 94°C for 30 s, T_A for 30 s, and 72°C for 30 s. The final extension step was at 72°C for 10 min. A 3 µl aliquot of each PCR product was analyzed on a 1.5% agarose gel and imaged using the Quantum-1100 imaging system and the Quantum Capt software (VWR, Darmstadt, Germany).

Purification and direct sequencing of PCR products

PCR products were validated by Sanger sequencing as follows: a 5 µl aliquot of the PCR product was treated with 10 U exonuclease I (Fermentas, St- Leon-Rot, Germany) and 1 U thermosensitive alkaline phosphatase (FastAP, Fermentas) in a final volume of 8 µl for 15 min at 37°C followed by inactivation at 80°C for 15 min. 2 µl of the respective *ExoI/FastAP* reaction were used for direct sequencing using the DYE-namic ET Terminator Cycle Sequencing Kit according to the manufacturer's protocol (GE Healthcare, Little Chalfont, United Kingdom). For sequence analysis, the MegaBACE 1000 sequencer (GE Healthcare) and Geneious software (version 5.3.6 created by Biomatters, available from

http://www.geneious.com) were used.

Genotyping controls

Spain: INfancia y Medio Ambiente

One thousand and seventy-one children whose parents were white and born in Spain or other European countries and that were not lost during the 4-year follow-up were selected for genotyping. Genome-wide genotyping was performed using the HumanOmni1-Quad v1.0 Beadchip (Illumina) at the Spanish National Genotyping Centre (CEGEN). Genotypes were called using the GeneTrain2.0 algorithm based on HapMap clusters implemented in the GenomeStudio software (Illumina). PLINK was used for genetic data quality control. We applied the following initial quality control thresholds: sample call rate > 98% and/or LRR SD < 0.3 (excluded: N=7: four from Valencia and two from Menorca, 0.7%). Next, we checked sex, relatedness (one duplicate sample and the younger brother of two brother-pairs in the INMA Sabadell cohort were excluded), heterozygosity, and population stratification. Genetic variants were filtered for SNP call rate > 95%, MAF > 1% and Hardy–Weinberg equilibrium (HWE) *P*-value > 1.10E-6. The final genetic data set consisted of 1,061 subjects from INMA Sabadell (N = 396), INMA Valencia (N = 349) and INMA Menorca (N = 316) and 817.131 QCed SNPs in b36 and + strand.

Imputation was performed with IMPUTEv2 using a cosmopolitan reference panel from the 1000 Genomes project (rel March 2012). After imputation, the database consisted of 39.346-413 SNPs (38.546.049 imputed and 800.364 genotyped).

Sweden SALT

The SALT individuals were genotyped using the Illumina OmniExpress platform and basic quality control metrics were applied at both SNP and sample levels. Briefly, SNPs with call rates < 95% and/or HWE $p < 10^{-5}$ were excluded. Samples were removed if: 1) call rates were < 98%, 2) genotype-imputed sex did not match the phenotype-reported sex, 3) they possessed an extreme heterozygosity rate (out of 3*SD of mean heterozygosity rate), and 4)

Formatat: espanyol (alfab. internacional)

they were related to other samples (PI-HAT > 0.1875), in which case the one with the highest missing rate in a related pair was removed.

Genome-wide imputation was then performed in two steps using Shapeit2 for phasing and IMPUTEv2 for imputation[5]. The 1000 genomes cosmopolitan population phase3 was used as a reference panel. Before imputation, all genotypes were aligned on the same strand with the reference panel using the "Genotype harmonizer" tool[6]. All SNPs that failed to align on the strand were excluded from further analyses. After imputation, we only kept SNPs with high imputation quality (info value of 0.8 and higher).

RNA extraction and reverse transcription

To quantify gene expression and to correlate differential expression driven by the SNP rs2020938 in P2, total RNA was extracted from various cell lines (HEK293T, Caco 2, Colo 320, SH-SY5Y, IMR-32) and from small and large intestine tissues (jejunum, ileum, colon, sigma) of IBS patients and control individuals using TRIZOL. One μg of total RNA was reverse transcribed into complementary DNA (cDNA) using the Superscript III-First-Strand-Synthesis-System (Invitrogen) as recommended by the manufacturer.

RT-PCR

Conventional RT-PCR from reverse transcribed cDNA was performed in 12.5 μl volumes containing 1–2 μl cDNA as template, 3.125 pmol of each primer, 5 pmol of each dNTP (MBI Fermentas), 1.25 μl HotStarTaq PCR Buffer 10x concentrated (contains Tris-HCl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$), and 0.625 U (0.125 μl) of HotStarTaq DNA Polymerase (Qiagen). Thermal cycling was performed in Mastercycler *vapo.protect* thermal cyclers (Eppendorf, Hamburg, Germany). Annealing temperatures (T_A) and sequences of specific *SLC6A4* expression primers are shown in Supplementary SD Table 5. Cycling conditions were: 15 min 95°C, followed by 40 cycles of 30 sec 94°C, 30 sec T_A, 30 sec 72°C, and a final

elongation for 10 min at 72°C. A 3 µl aliquot of each PCR product was analyzed on a 1.5 % agarose gel and imaged using the Quantum-1100 Imaging System and the Quantum Capt software (VWR).

Quantitative PCR (qPCR)

Relative gene expression was analyzed by qPCR on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, California) according to the manufacturer's instructions. Forward and reverse primers and TaqMan probes specific for *SLC6A4* P1 and P2 promoters were designed according to standard guidelines and their relative efficiency tested as described in qPCR Application Guide Experimental Overview, Protocol, Troubleshooting. Third Edition (https://www.gene-quantification.de/idt-miqe-qpcrguide-2010.pdf). Primer sequences are specified in Supplementary SD Table 5. All values were normalized to 18 S RNA (Applied Biosystems, TaqMan Assay ID: Hs99999901_s1). Each sample, including sterile distilled water as a negative control, was run in triplicate and data were analyzed by the 2-ΔΔCt method with correction for primer efficiency.

nCounter analysis

The jejunum was the only tissue with robust P2-driven SERT expression, so nCounter targeted gene expression analysis (nanoString Technologies, Seattle, WA, USA) was carried out on jejunal samples. The nCounter technology allows for multiplexed gene expression analysis based on simultaneous hybridization and digital quantification of fluorescently labeled oligonucleotide probes[7]. A reporter probe which carries a fluorescent barcode, a biotinylated capture probe (elements tag set) that immobilizes the complex for data collection, and two target gene-specific oligonucleotide probes A+B (probe set) are necessary to detect each transcript (Supplementary SD Table 6).

All RNA samples were quantified by using the Qubit™ RNA HS assay kit and quality was controlled using the Agilent RNA6000 Nanokit on an Agilent 2100 Bioanalyzer system.

Qualifying samples were subjected to nCounter analysis as recommended by the manufacturer. In brief, one hundred ng of total RNA were used as input material for Elements probe set hybridization at 65°C. The experiment was read out using the SPRINT™ Profiler from nanoString Technologies. Data were normalized and evaluated using the nSolver Analysis Software (version 2.0) provided by nanoString Technologies (https://www.nanostring.com/products/analysis-software/nsolver). Stably expressed reference genes were chosen for normalization based on the Normfinder method[8].

Transfections

To investigate the functional effect of the most contrasting major and minor P2 promoter haplotypes on gene transcription, the human embryonic kidney cell line HEK292T, the human neuroblastoma cell line SH-SY5Y, the colorectal carcinoma cell lines Caco 2 and Colo 320 cells were seeded into 6-well plates with approximately 40–70% cell density in Opti-MEM I Reduced Serum Media + 10 % FBS (both Thermo Fisher Scientific) one day prior to transfection. All cell lines but SH-SY5Y were transfected with Polyethylenimin (PEI, Sigma Aldrich) applying a ratio of 1:4 (1 µg DNA: 4 µg PEI). SH-SY5Y cells were transfected using Lipofectamine 2000 (Thermo Fisher Scientific) as recommended by the manufacturer. 24 hours post-transfection, cells were lysed in 100 µl 1x Passive Lysis buffer (Promega, Madison, Wisconsin) per reaction. Lysed cells were frozen at -80°C for one hour and dissolved in lysis buffer prior to luciferase assay.

Luciferase reporter assays

For luciferase assays, *SLC6A* P2 promoter constructs were made using pGL3 basic vectors (Promega). The two major haplotype version based promoters were cloned upstream via *XhoI* and *HindIII* (Supplementary SD Table 7). Reporter gene constructs used were pGL3 basic vector (with and without upstream P2 region insert), the pGL3 control vector (both Promega), and PRL-TK-Vector (reference construct for normalization). 25 µl of each cell

lysate was placed in each well of a 96-well plate as input material for the luciferase assay. The assay was performed using the Dual-Luciferase Reporter Assay Systems (Promega) on a Lucy2 luminometer (Rosys Anthos Mikrosysteme, Krefeld, Germany) as recommended by the manufacturer.

Transcription factor binding site prediction

Transcription factor binding sites of the promoter P2 region containing the SNPs including rs2020938 linked in the major and minor haplotype were compared using the online tool ePOSSUM2 (https://www.mutationdistiller.org/ePOSSUM2/). The input sequences were as follows.

Risk – major haplotype:

aaagcctatcaagcctagttgagggcccaaggaaggggggaagtgggggggaggccaaggccaggcagtagcataaatggtg
agcagggtgaggttatggagatcgctcttgtcagaattcatatccttctgagtggtgtttgcattcttgagcctggggtgggtggtgga
gggcccaacaagtgtggtacagcccaagacg ;

Protective - minor haplotype :

aaagcctatcaagcctagttgagggccgaaggaaggggggaagtgggggggaggccaaggccaggcagtagcataaatggtg
agcagggtgaggttatggagaccgctcttgtcagaattcatatccttctgagtggtgtttgcattcttgagcctggggtgggtggcgga
gggcccaacaagtgcggtacagcccaagacg

This tool predicts the impact of DNA variants on transcription factor binding. ePOSSUM2 supports more than 1,000 different binding models for 247 human transcription factors. The models were pooled from various publicly accessible data sources, such as CIS-BP, Hocomoco, hPDI, JASPAR, and Jolma (2013). Overall, 103 transcription factor flexible models are included. For each binding model, ePOSSUM2 constructs a Bayes classifier and reports the likelihood that a variant leads to the gain or loss of a transcription factor binding site. Because several binding models detected experimentally verified sites poorly, a negative or positive predictive value is reported. ePOSSUM2 was developed in 2018 at the Berlin Institute of Health (BIH) and Charité – Universitätsmedizin Berlin by Robin Steinhaus, Peter N. Robinson, and Dominik Seelow. ePOSSUM2 is an update of the ePOSSUM software.

In order to collect further evidence and validate ePOSSUM predictions, we explored of ChIP Atlas data (https://chip-atlas.org/search) for transcription factors binding in the corresponding promoter region of *SLC6A4*.

Ingenuity pathway analysis (IPA)

Pathway and network analysis was performed using IPA software (Ingenuity® Systems, https://www.qiagenbioinformatics.com/). IPA integrates gene data sets with all the biological information available in public databases to predict the functional biological context (biological functions and signaling pathways). For network analysis, IPA provides a score according to the fit of supplied genes and the list of biological functions involved. Core analysis was performed on the 52 transcription factors predicted by ePOSSUM2.

Statistical analysis for luciferase assays and qPCR analysis

Two-tailed parametric tests were used as appropriate (unpaired *t*-test, one-way ANOVA followed by Bonferroni correction post-hoc test) using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla; California). A Mann-Whitney *U* test and an unpaired t-test with Welch's correction were applied when data did not follow a normal distribution, as indicated in the figure legends.

Relationships between clinical features (bowel movement, stool form) and qPCR-based mRNA expression levels were assessed by Spearman's correlation rho. Data are expressed as mean ± standard error of the mean, unless stated otherwise. *P*-values of < 0.05 ($*P < 0.05$) were considered statistically significant.

Luciferase data analysis was performed using GraphPad Prism 8.4.2 software applying unpaired multiple t tests of all data. Discovery was determined using the two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli.

SD Results

SLC6A4 expression analysis in different cell lines

To select appropriate cell lines for functional follow-up studies of the variant promoter P2 haplotypes, we assessed the expression profile of S*LC6A4* in various cell lines (SH-SY5Y, IMR-32, Caco 2, HEK293T, and Colo 320 cells; Supplementary SD Figure 2). For this purpose, we isolated total RNA and generated cDNAs for RT-PCR analysis. All analyzed cell lines expressed *SLC6A4* and were therefore all selected for functional analysis of the major and minor haplotypes of promoter P2 by luciferase reporter assays except for IMR-32.

SD Results Tables

SD Table 8 Detected variants in promoter region P2, *GnomAD, na not annotated

rs number	Minor Allele	MAF	MAF dbSNP*	Position GRCh38.p12	Variant	*SLC6A4* RefSeqGene
rs12150214	G	0.2	0.25	chr17:30223870	NC_000017.11:g.30223870C>G	NG_011747.2:g.17067G>C
rs2020936	C	0.2	0.24	chr17:30223796	NC_000017.11:g.30223796G>A	NG_011747.2:g.17141C>T
rs2020937	A	0.39	0.35	chr17:30223739	NC_000017.11:g.30223739A>T	NG_011747.2:g.17198T>A
rs2020938	C	0.2	0.44	chr17:30223732	NC_000017.11:g.30223732G>A	NG_011747.2:g.17205C>T
rs2020939	T	0.0398	0.35	chr17:30223714	NC_000017.11:g.30223714G>A	NG_011747.2:g.17223C>T
na	T	0.0017	na	chr17:30223679	NC_000017.11:g.30223679C>T	NG_011747.2:g.17188C>T
na	T	0.0017	na	chr17:30222976	NC_000017.11:g.30222976C>T	NG_011747.2:g.17989C>T
rs2020940	C	0.077	0.018	chr17:30222962	NC_000017.11:g.30222962C>G	NG_011747.2:g.17975G>C
rs25528	C	0.199	0.28	chr17:30222960	NC_000017.11:g.30222960G>T	NG_011747.2:g.17977C>A
rs2020941	A	0.021	0.001	chr17:30222940	NC_000017.11:g.30222940C>T	NG_011747.2:g.17997G>A
rs6354	C	0.2	0.22	chr17:30222880	NC_000017.11:g.30222880G>T	NG_011747.2:g.18057C>A
rs28914827	A	0.0297	0.012	chr17:30222791	NC_000017.11:g.30222791C>T	NG_011747.2:g.18146G>A

SD Table 9 Test for deviation from Hardy–Weinberg Equilibrium (*P*-value) in patient and control samples

SLC6A4	**rs2020938**				
Study cohort	IBS overall	IBS-C	controls	female IBS-C	female controls
UK1	0.31	0.027	0.26	0.041	0.74
Germany1	0.39	1.00	1.00	1.00	1.00
Germany2	0.33	0.50	0.45	0.54	0.57
UK2+3	0.63	0.73	1.00	0.70	0.76
Ireland	0.56	1.00	0.42	1.00	0.65
USA1	1.00	1.00	0.43	1.00	1.00
USA2	0.88	0.82	1.00	1.00	0.75
USA3	0.47	0.064	0.49	0.17	1.00
Sweden	0.43	1.00	1.00	1.00	0.30
Spain	1.00	n.a.	0.22	n.a.	0.39
Greece	1,65E-12	1,65E-12	0.065	1,68E-12	0.17
Chile	0.74	1.00	1.00	1.00	1.00

370 **SD Table 10** Ct values of qPCR data *SLC6A4* P1/P2 driven isoforms
371

Sample code	Group	Tissue	18s Ct	*SLC6A4* P1 Ct	*SLC6A4* Ct
ER-MK-MR-003	IBS	Ileum	16,57833337	35,50196707	24,89783242
ER-MK-MR-004	IBS	Ileum	18,76806721	38,74713988	27,17786836
ER-MK-MR-007	IBS	Ileum	15,78976814	33,12196345	23,75244634
ER-MK-MR-009	IBS	Ileum	15,1460357	32,19460727	22,81192444
ER-MK-MR-010	IBS	Ileum	15,46972902	33,072317	23,55361965
ER-MK-MR-011	IBS	Ileum	15,3973569	35,74640249	25,45528824
ER-MK-MR-012	IBS	Ileum	15,69719769	34,74001429	24,84114996
ER-MK-MR-013	IBS	Ileum	16,87498135	35,16516928	25,75574305
ER-MK-MR-015	IBS	Ileum	15,98474015	34,61576191	23,25792871
ER-MK-MR-017	IBS	Ileum	15,62578989	34,0614149	24,18704425
ER-MK-MR-019	IBS	Ileum	15,75802725	35,07133089	23,30192139
ER-MK-MR-020	IBS	Ileum	16,62464062	34,38156678	23,74253981
ER-MK-MR-021	IBS	Ileum	16,23376757	32,30141317	23,7278778
ER-MK-MR-025	IBS	Ileum	17,22877481	35,8002207	25,25428307
ER-MK-MR-026	IBS	Ileum	16,24136959	35,13770016	23,90798373
ER-MK-MR-027	IBS	Ileum	15,73517447	33,38682527	23,23383261
ER-MK-MR-028	IBS	Ileum	16,32410269	34,58312108	23,7072518
ER-MK-MR-001	Healthy control	Ileum	15,36049295	35,90217299	27,2274689
ER-MK-MR-006	Healthy control	Ileum	15,61590827	33,93056589	23,76490999
ER-MK-MR-008	Healthy control	Ileum	15,29043582	33,64500865	24,22376062
ER-MK-MR-016	Healthy control	Ileum	15,44596624	32,98832515	23,42277352
ER-MK-MR-022	Healthy control	Ileum	16,31101889	33,13384236	23,38881028
ER-MK-MR-023	Healthy control	Ileum	16,25127578	34,56713914	23,66936656
ER-MK-MR-024	Healthy control	Ileum	16,45577012	38,22063153	25,88062652
ER-MK-MR-029	Healthy control	Ileum	16,01129456	33,42104593	23,33013675
ER-MK-MR-030	Healthy control	Ileum	15,93677023	34,05102097	23,43950417
BCN2	IBS	Jejunum	18,34616089	39,73058166	30,81976709
BCN3	IBS	Jejunum	17,23204803	37,34487938	28,94345808
BCN5	IBS	Jejunum	17,95299339	38,33751658	30,32406309
BCN6	IBS	Jejunum	17,66653442	37,72347639	30,7431139
BCN7	IBS	Jejunum	16,87281036	37,535122	28,7124546
BCN16	IBS	Jejunum	17,40193558	35,97644571	28,0237678
BCN17	IBS	Jejunum	17,0983696	35,45454652	27,2499728
BCN18	IBS	Jejunum	17,74133682	34,39541101	27,35790818
BCN19	IBS	Jejunum	17,19239807	36,32251025	28,4785045
BCN42	IBS	Jejunum	17,55171204	36,59863221	28,46095552
BCN43	IBS	Jejunum	17,61321068	35,55451009	27,49397446
BCN44	IBS	Jejunum	17,00183296	34,56787078	27,78468358
BCN45	IBS	Jejunum	18,14543343	37,32597404	29,11618248
BCN46	IBS	Jejunum	19,03618622	undetermined	29,4530488
BCN47	IBS	Jejunum	18,01171875	35,10915844	27,05952716
BCN48	IBS	Jejunum	16,81947327	35,40755294	27,68018541
BCN49	IBS	Jejunum	17,3154335	34,56916434	27,29516354

BCN50	IBS	Jejunum	17,83892822	37,95594248	28,89515524
BCN51	IBS	Jejunum	18,1	35,34533878	29,22341294
BCN52	IBS	Jejunum	18,32463455	38,31718656	29,75942126
BCN53	IBS	Jejunum	18,49115372	37,71374177	29,60427638
BCN54	IBS	Jejunum	17,05583572	34,30906855	27,01303968
BCN55	IBS	Jejunum	17,57375336	35,00560603	28,07346943
BCN56	IBS	Jejunum	17,06829071	37,18562865	30,42199954
BCN57	IBS	Jejunum	17,04096794	33,59088479	27,14901846
BCN58	IBS	Jejunum	16,96086884	35,70972135	27,49031071
BCN59	IBS	Jejunum	17,35703278	36,67221818	27,5330794
BCN60	IBS	Jejunum	16,87817001	38,15106697	27,78926574
BCN61	IBS	Jejunum	16,86527443	35,32750925	27,15187509
BCN62	IBS	Jejunum	16,55615997	35,38464389	27,88648716
BCN63	IBS	Jejunum	17,13666344	35,46373373	28,07083258
BCN64	IBS	Jejunum	16,91453362	35,33075682	27,13665483
BCN70	IBS	Jejunum	18,97595406	37,41222347	30,45523216
BCN71	IBS	Jejunum	18,5400238	39,77472022	30,72117842
BCN72	IBS	Jejunum	16,72651863	36,78650715	27,81226619
BCN73	IBS	Jejunum	19,53974533	undetermined	31,12689689
BCN74	IBS	Jejunum	17,49632454	38,26791168	28,55567605
BCN28	Healthy control	Jejunum	16,84288406	36,49087586	28,4539809
BCN34	Healthy control	Jejunum	17,50685883	37,7218735	29,86843379
BCN35	Healthy control	Jejunum	17,85357285	36,46522095	28,58128621
BCN36	Healthy control	Jejunum	17,57317352	35,45570037	28,26737634
BCN37	Healthy control	Jejunum	17,87538147	37,72672186	27,61536111
BCN38	Healthy control	Jejunum	17,77909851	36,77850621	28,74544825
BCN39	Healthy control	Jejunum	16,60348129	35,62351994	27,77497335
BCN40	Healthy control	Jejunum	16,90099335	34,6715074	28,05497559
BCN41	Healthy control	Jejunum	18,10161018	35,58449196	28,41336675
BCN68	Healthy control	Jejunum	16,5283432	33,76212561	26,99324395
BCN69	Healthy control	Jejunum	17,0928421	35,06656377	26,82039283
ER-MK-MR-007	IBS	Sigma	17,44391868	34,29	35,26
ER-MK-MR-009	IBS	Sigma	15,43060381	Undertemined	32,51101748
ER-MK-MR-010	IBS	Sigma	15,51610788	36,32457542	33,01600647
ER-MK-MR-011	IBS	Sigma	16,34921776	Undertemined	35,95563889
ER-MK-MR-012	IBS	Sigma	16,68834373	Undertemined	37
ER-MK-MR-013	IBS	Sigma	16,40824573	36,99	35,12139384
ER-MK-MR-014	IBS	Sigma	16,13374419	Undertemined	36,94646962
ER-MK-MR-015	IBS	Sigma	16,00693323	35,41	34,66649628
ER-MK-MR-017	IBS	Sigma	16,02373634	36,55	37,24346542
ER-MK-MR-018	IBS	Sigma	16,59000327	Undertemined	34,03622818
ER-MK-MR-019	IBS	Sigma	15,55161112	37,1	34,81573232
ER-MK-MR-021	IBS	Sigma	16,00698701	Undertemined	35,36591085
ER-MK-MR-025	IBS	Sigma	16,27040304	36,2	31,30365817
ER-MK-MR-008	Healthy control	Sigma	15,87784914	35,15	31,0500164
ER-MK-MR-016	Healthy control	Sigma	16,38361487	Undertemined	31,99977748

ER-MK-MR-022	Healthy control	Sigma	17,29106473	36,48316193	34,16260529
ER-MK-MR-023	Healthy control	Sigma	16,13667951	35,6	33,66689555
ER-MK-MR-024	Healthy control	Sigma	17,29880359	Undertemined	32,77197393
2	IBS	Colon	18,29960962	Undetermined	32,87808355
5	IBS	Colon	17,20583263	Undetermined	35,04758708
6	IBS	Colon	16,3521575	Undetermined	31,46317927
13	IBS	Colon	17,55937758	36,94776535	32,295976
14	IBS	Colon	16,88134367	Undetermined	30,15899531
19	IBS	Colon	17,08260609	36,56434441	31,34770012
20	IBS	Colon	16,78699822	Undetermined	31,73283323
22	IBS	Colon	19,54240328	Undetermined	32,46275965
23	IBS	Colon	17,29031609	36,63349724	30,70471509
24	IBS	Colon	17,01536734	Undetermined	36,97
26	IBS	Colon	17,93946522	35,74	30,5747172
27	IBS	Colon	16,8949637	Undetermined	30,973032
28	IBS	Colon	17,35046852	Undetermined	30,70275688
29	IBS	Colon	16,4955049	35,54	30,66182264
30	IBS	Colon	17,31728934	35,84172058	30,76026217
33	IBS	Colon	17,05520735	Undetermined	32,4541022
34	IBS	Colon	17,53008811	Undetermined	31,86995443
35	IBS	Colon	18,39402905	36,37	30,52995173
36	IBS	Colon	16,67222367	36,94	30,51552773
38	IBS	Colon	17,07300162	Undertemined	30,8369058
39	IBS	Colon	18,57020071	Undertemined	32,13900566
3	Healthy control	Colon	16,81724649	Undetermined	31,96453094
7	Healthy control	Colon	17,34802874	Undetermined	31,65384229
8	Healthy control	Colon	16,91298886	35,25	33,52826436
9	Healthy control	Colon	17,08674982	36,3321209	31,08000628
10	Healthy control	Colon	16,8024042	Undetermined	31,54023679
15	Healthy control	Colon	17,20890205	Undetermined	32,75512441
16	Healthy control	Colon	17,75628429	Undetermined	36,59430122
17	Healthy control	Colon	17,18627134	Undetermined	33,67042414
18	Healthy control	Colon	17,38047673	Undetermined	31,8262469
21	Healthy control	Colon	17,08621864	Undetermined	30,93513743
25	Healthy control	Colon	16,57395005	35,83358256	30,62000529
31	Healthy control	Colon	17,56393995	Undetermined	30,31180064
32	Healthy control	Colon	17,0698365	36,83	32,20366542
37	Healthy control	Colon	18,23812589	35,62	30,45488739

372

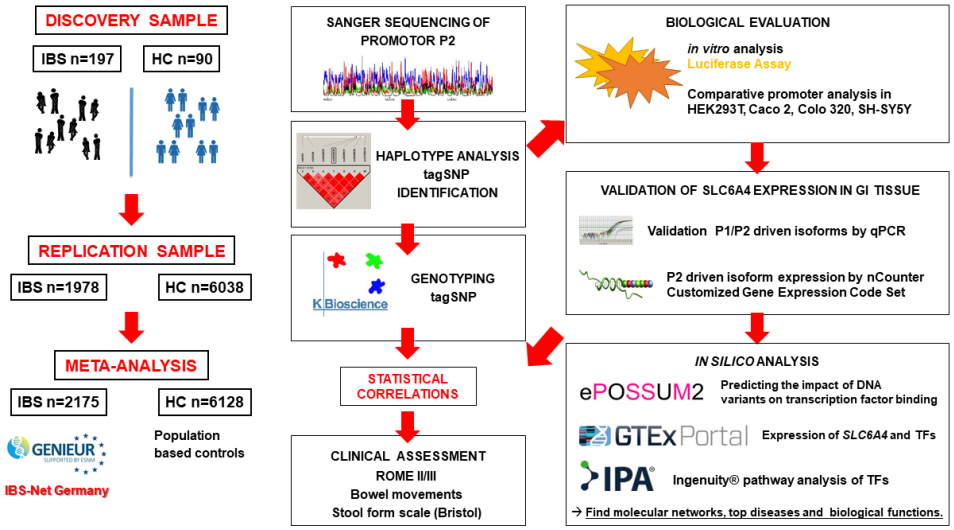
SD Table 11 ePOSSUM2 predicted transcription factors with gained binding sites in the polymorphic minor haplotype of the P2 promoter region and corresponding ChIP atlas data. TFs not included in ChIP Atlas data are indicated in grey.

Symbol	Entrez Gene Name	Type(s)	Entrez Gene ID for Human	ChIP Atlas data
ATF1	activating transcription factor 1	transcription regulator	466	no
ATF2	activating transcription factor 2	transcription regulator	1386	no
ATF7	activating transcription factor 7	transcription regulator	11016	no
BATF	basic leucine zipper ATF-like transcription factor	transcription regulator	10538	no
CEBPA	CCAAT enhancer binding protein alpha	transcription regulator	1050	no
CHD2	chromodomain helicase DNA binding protein 2	enzyme	1106	no
CREB3L1	cAMP responsive element binding protein 3 like 1	transcription regulator	90993	no
CREM	cAMP responsive element modulator	transcription regulator	1390	no
E4F1	E4F transcription factor 1	transcription regulator	1877	no
ELF3	E74 like ETS transcription factor 3	transcription regulator	1999	no
ELF4	E74 like ETS transcription factor 4	transcription regulator	2000	no
ETS1	ETS proto-oncogene 1, transcription factor	transcription regulator	2113	hESC_derived_cardiac_cells
FOSL1	FOS like 1, AP-1 transcription factor subunit	transcription regulator	8061	no
FOXA1	forkhead box A1	transcription regulator	3169	293, DU145 (prostate cancer cell line), HepG2 (liver cancer), mesendodermal cells, Ishikawa (endometrial adenocarcinoma), LNCaP (prostate carcinoma), MCF-7 (breast carcinoma), MDA-MB-453 (breasr cancer), NCI-H3122 (lung adenocarcinoma), Prostatic_Neoplasms, T-47D (breast cancer), VCaP (prostate cancer), ZR-75-1 (breast cancer)
FOXA2	forkhead box A2	transcription regulator	3170	HepG2 (liver cancer), hESC_derived_mesendodermal_cells, LNCaP (prostate carcinoma)
FOXA3	forkhead box A3	transcription regulator	3171	not contained in ChiP Atlas
FOXP1	forkhead box P1	transcription regulator	27086	OCI-LY1 (B cell lymphoma)
GATA4	GATA binding protein 4	transcription regulator	2626	no
GLIS1	GLIS family zinc finger 1	transcription regulator	148979	not contained in ChiP Atlas
GLIS2	GLIS family zinc finger 2	transcription regulator	84662	not contained in ChiP Atlas
GMEB1	glucocorticoid modulatory element binding protein 1	transcription regulator	10691	not contained in ChiP Atlas
GMEB2	glucocorticoid modulatory element binding protein 2	transcription regulator	26205	no
HMBOX1	homeobox containing 1	transcription regulator	79618	no
HNF1A	HNF1 homeobox A	transcription regulator	6927	no
HNF4G	hepatocyte nuclear factor 4 gamma	transcription regulator	3174	no
IRF4	interferon regulatory factor 4	transcription regulator	3662	no
JUND	JunD proto-oncogene, AP-1 transcription factor subu	transcription regulator	3727	no
KLF7	Kruppel like factor 7	transcription regulator	8609	not contained in ChiP Atlas
MEF2A	myocyte enhancer factor 2A	transcription regulator	4205	no
MITF	melanogenesis associated transcription factor	transcription regulator	4286	Hermes-3C (melanocyte cell line)
MYBL2	MYB proto-oncogene like 2	transcription regulator	4605	no
NFE2L2	nuclear factor, erythroid 2 like 2	transcription regulator	4780	no
NFIB	nuclear factor I B	transcription regulator	4781	no
NFIL3	nuclear factor, interleukin 3 regulated	transcription regulator	4783	not contained in ChiP Atlas
NR2F2	nuclear receptor subfamily 2 group F member 2	ligand-dependent nuclear receptor	7026	no
NR2F6	nuclear receptor subfamily 2 group F member 6	ligand-dependent nuclear receptor	2063	no
NR3C1	nuclear receptor subfamily 3 group C member 1	ligand-dependent nuclear receptor	2908	no
RAD21	RAD21 cohesin complex component	transcription regulator	5885	(HEK293 Bax Bak double knock-out), ECC-1 (endometrial cancer) , GM12878 (lymphoblastoid cell line), GP5d (adenocarcinoma), **HCT-116 (colon cancer cell line),** HEC-1-B (endometrium adenocarcinoma), HeLa, HepG2 (liver cancer), hESC_H1, hESC_H9, hESC_HUES64 (human embryonic stem cell lines =hESC) , HUVEC (primary endothelial cells), K-562 (leukemia cell line), **LoVo (colon adenocarcinoma)**, Macrophages, MCF-7 (breast carcinoma),
RFX5	regulatory factor X5	transcription regulator	5993	
RXRB	retinoid X receptor beta	ligand-dependent nuclear receptor	6257	not contained in ChiP Atlas
SPI1	Spi-1 proto-oncogene	transcription regulator	6688	(liver cancer), TALL-1 (leukemia cell line)
SREBF1	sterol regulatory element binding transcription factor	transcription regulator	6720	no
STAT2	signal transducer and activator of transcription 2	transcription regulator	6773	no
TCF7	transcription factor 7	transcription regulator	6932	no
TCF7L2	transcription factor 7 like 2	transcription regulator	6934	no
THRA	thyroid hormone receptor alpha	ligand-dependent nuclear receptor	7067	no
THRB	thyroid hormone receptor beta	ligand-dependent nuclear receptor	7068	not contained in ChiP Atlas
ZBTB49	zinc finger and BTB domain containing 49	transcription regulator	166793	not contained in ChiP Atlas
ZBTB7A	zinc finger and BTB domain containing 7A	transcription regulator	51341	2 (human umbilical cord blood-derived erythroid progenitor),
ZNF24	zinc finger protein 24	transcription regulator	7572	not contained in ChiP Atlas
ZNF652	zinc finger protein 652	other	22834	not contained in ChiP Atlas
ZSCAN16	zinc finger and SCAN domain containing 16	transcription regulator	80345	not contained in ChiP Atlas

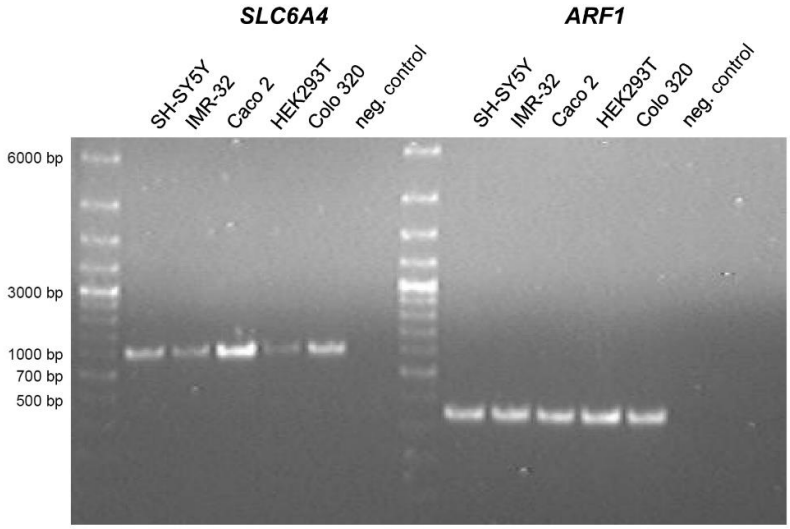
SD Table 12 GTEx eQTL data rs2020938 (Single-Tissue eQTLs for chr17_30222791_C_T_b38; Data Source: GTEx Analysis Release V8 (dbGaP Accession phs000424.v8. p2))

Gencode Id	Gene Symbol	Variant Id	SNP Id	P-Value	NES	Tissue
ENSG00000176927.15	*EFCAB5*	chr17_30223732_G_A_b38	rs2020938	5.3e-8	0.35	Cells - Cultured fibroblasts
ENSG00000167549.18	*CORO6*	chr17_30223732_G_A_b38	rs2020938	8.4e-8	0.30	Thyroid
ENSG00000176927.15	*EFCAB5*	chr17_30223732_G_A_b38	rs2020938	3.3e-7	0.31	Thyroid
ENSG00000231421.7	*AC006050.2*	chr17_30223732_G_A_b38	rs2020938	8.2e-7	-0.57	Brain - Hippocampus
ENSG00000141298.17	*SSH2*	chr17_30223732_G_A_b38	rs2020938	0.0000063	0.059	Whole Blood
ENSG00000264538.6	*SUZ12P1*	chr17_30223732_G_A_b38	rs2020938	0.0000066	0.36	Brain - Caudate (basal ganglia)
ENSG00000231421.7	*AC006050.2*	chr17_30223732_G_A_b38	rs2020938	0.0000067	-0.53	Brain - Amygdala
ENSG00000231421.7	*AC006050.2*	chr17_30223732_G_A_b38	rs2020938	0.0000070	-0.57	Brain - Spinal cord (cervical c-1)
ENSG00000264538.6	*SUZ12P1*	chr17_30223732_G_A_b38	rs2020938	0.000010	0.16	Lung
ENSG00000231421.7	*AC006050.2*	chr17_30223732_G_A_b38	rs2020938	0.000011	-0.46	Brain - Nucleus accumbens (basal ganglia)
ENSG00000176927.15	*EFCAB5*	chr17_30223732_G_A_b38	rs2020938	0.000013	0.28	Adipose - Subcutaneous
ENSG00000264538.6	*SUZ12P1*	chr17_30223732_G_A_b38	rs2020938	0.000014	0.20	Adipose - Subcutaneous
ENSG00000231421.7	*AC006050.2*	chr17_30223732_G_A_b38	rs2020938	0.000014	-0.46	Ovary
ENSG00000231421.7	*AC006050.2*	chr17_30223732_G_A_b38	rs2020938	0.000023	-0.39	Brain - Cortex
ENSG00000141298.17	*SSH2*	chr17_30223732_G_A_b38	rs2020938	0.000031	0.11	Nerve - Tibial
ENSG00000263531.1	*RP13-753N3.1*	chr17_30223732_G_A_b38	rs2020938	0.000035	-0.32	Cells - Cultured fibroblasts
ENSG00000264538.6	*SUZ12P1*	chr17_30223732_G_A_b38	rs2020938	0.000037	0.20	Nerve - Tibial
ENSG00000231421.7	*AC006050.2*	chr17_30223732_G_A_b38	rs2020938	0.000046	-0.38	Brain - Cerebellum
ENSG00000264538.6	*SUZ12P1*	chr17_30223732_G_A_b38	rs2020938	0.000056	0.17	Skin - Sun Exposed (Lower leg)
ENSG00000172171.10	*TEFM*	chr17_30223732_G_A_b38	rs2020938	0.000090	0.12	Muscle - Skeletal
ENSG00000108262.15	*GIT1*	chr17_30223732_G_A_b38	rs2020938	0.00017	-0.096	Whole Blood
ENSG00000176390.11	*CRLF3*	chr17_30223732_G_A_b38	rs2020938	0.00019	0.14	Artery - Tibial
ENSG00000264538.6	*SUZ12P1*	chr17_30223732_G_A_b38	rs2020938	0.00020	0.18	Artery - Tibial
ENSG00000264538.6	*SUZ12P1*	chr17_30223732_G_A_b38	rs2020938	0.00021	0.18	Cells - Cultured fibroblasts
ENSG00000263603.1	*CTD-2349P21.*	chr17_30223732_G_A_b38	rs2020938	0.00023	0.25	Cells - Cultured fibroblasts
ENSG00000176927.15	*EFCAB5*	chr17_30223732_G_A_b38	rs2020938	0.00032	0.21	Skin - Sun Exposed (Lower leg)

SD Figures



SD Figure 1 Summary of experimental design. Workflow of the analysis performed on biospecimens of human subjects and complementing *in vitro* and *in silico* analyses into the functional relevance of detected variants.



391
392
393 **SD Figure 2** *SLC6A4* expression validation in SH-SY5Y, IMR-32, Caco 2, HEK293T, and
394 Colo 320 cells. *ARF1* was included as positive control for cDNA integrity. Marker: Gene
395 Ruler™1kb DNA ladder (MBI Fermentas). Note: IMR-32 was not further included in analyses.

SD References

1. Wohlfarth C, Schmitteckert S, Hartle JD, et al. miR-16 and miR-103 impact 5-HT4 receptor signalling and correlate with symptom profile in irritable bowel syndrome. *Sci Rep*. Oct 31 2017;7(1):14680. https://doi.org10.1038/s41598-017-13982-0.
2. Ek WE, Reznichenko A, Ripke S, et al. Exploring the genetics of irritable bowel syndrome: a GWA study in the general population and replication in multinational case-control cohorts. *Gut*. Nov 2015;64(11):1774-82. https://doi.org10.1136/gutjnl-2014-307997.
3. Wouters MM, Lambrechts D, Knapp M, et al. Genetic variants in CDC42 and NXPH1 as susceptibility factors for constipation and diarrhoea predominant irritable bowel syndrome. *Gut*. Jul 2014;63(7):1103-11. https://doi.org10.1136/gutjnl-2013-304570.
4. Guxens M, Ballester F, Espada M, et al. Cohort Profile: the INMA--INfancia y Medio Ambiente--(Environment and Childhood) Project. *Int J Epidemiol*. Aug 2012;41(4):930-40. https://doi.org10.1093/ije/dyr054.
5. van Leeuwen EM, Kanterakis A, Deelen P, et al. Population-specific genotype imputations using minimac or IMPUTE2. *Nat Protoc*. Sep 2015;10(9):1285-96. https://doi.org10.1038/nprot.2015.077.
6. Deelen P, Bonder MJ, van der Velde KJ, et al. Genotype harmonizer: automatic strand alignment and format conversion for genotype data integration. *BMC Res Notes*. Dec 11 2014;7:901. https://doi.org10.1186/1756-0500-7-901.
7. Geiss GK, Bumgarner RE, Birditt B, et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nat Biotechnol*. Mar 2008;26(3):317-25. https://doi.org10.1038/nbt1385.
8. Vandesompele J, De Preter K, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol*. Jun 18 2002;3(7):RESEARCH0034.

Acknowledgements

We thank Dr. Theadore Ptak, Renee Henry, Ellen Goldstein, Cindy Lee, Deborah Roach, Jacqueline Rabuzin and Elizabeth Crosland (Toronto Digestive Disease Associates (TDDA Inc.), Toronto, Ontario, Canada), Dr. Mark Silverberg and Lori Baladjay (Mount Sinai Hospital, Toronto, Ontario, Canada), Dr. Yehuda Ringel, Dr. Robert Sandler, Alesia N. Aileo, Sarah Causey and Sarah Yeskel (University of North Carolina at Chapel Hill, NC, USA) as well as the staff at Ersta Hospital (Stockholm, Sweden) for collecting samples and acquiring data and Dr. Rachel Gibson and Dr. George Dukes (GlaxoSmithKline) for logistic support as well as for patient recruitment and clinical data collection.

Thanks to Milagros Gallart, Adoración Nieto, Laura Hernández and Sara Méndez from Hospital Universitari Vall d'Hebron for their invaluable assistance in the performance of jejunal biopsies. GB is supported by a grant from the Flanders Research Foundation (FWO,

Odysseus). MW was supported by a FWO postdoctoral research fellowship. MD was funded by the Swedish Research Council (VR). APC Microbiome Ireland is funded by Science Foundation Ireland (SFI), through the Irish Government's National Development Plan. The authors and their work were supported by SFI (grant number SFI/12/RC/2273 P2) and by the Health Research Board through Health Research Awards (grant no HRA_POR/2011/23; TGD, JFC, and GC). MS is supported by the Swedish Medical Research Council (grants 13409, 21691 and 21692), the Marianne and Marcus Wallenberg Foundation, and the University of Gothenburg, Centre for Person-Centred Care (GPCC), Sahlgrenska Academy, University of Gothenburg and by the Faculty of Medicine, University of Gothenburg; MV and JS are supported by Fondo de Investigación Sanitaria and CIBERehd, Instituto de Salud Carlos III, Subdirección General de Investigación Sanitaria, Ministerio de Economía y Competitividad (PI13/00935, PI14/00994); CM is supported by Instituto de Salud Carlos III, Subdirección General de Investigación Sanitaria, Ministerio de Ciencia, Innovación y Universidades (CP18/00116). The UK Nottingham cohort was supported by a Research for Patient Benefit grant and salary support for Dr. Garsed from the Nottingham Digestive Diseases Biomedical Research Unit. The Chilean Santiago cohort was supported by FONDECYT No11121527, No1181699.

The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health as well as by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. The data used for the analyses described in this manuscript were obtained from the GTEx portal (Release v08 on 26 January 2021).