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A GWAS meta-analysis from 5 population-based cohorts implicates ion channel genes in the pathogenesis of irritable bowel syndrome

F. Bonfiglio[1,2] | M. Henström[2] | A. Nag[3] | F. Hadizadeh[2] | T. Zheng[2] | M. C. Cenit[4] | E. Tigchelaar[4] | F. Williams[3] | A. Reznichenko[2] | W. E. Ek[2,5] | N. V. Rivera[2] | G. Homuth[6] | A. A. Aghdassi[7] | T. Kacprowski[6] | M. Männikkö[8] | V. Karhunen[8,9,10] | L. Bujanda[1,11] | J. Rafter[2] | C. Wijmenga[4] | J. Ronkainen[8,12] | P. Hysi[13] | A. Zhernakova[4] | M. D'Amato[1,14,15,16]

[1]Department of Gastrointestinal and Liver Diseases, Biodonostia Health Research Institute, Spain

[2]Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden

[3]Department of Twin Research & Genetic Epidemiology, King's College London, London, England

[4]Department of Genetics, University Medical Center Groningen, Groningen, The Netherlands

[5]Department of Immunology, Genetics and Pathology, Science for Life Laboratory Uppsala, Uppsala University, Uppsala, Sweden

[6]Department of Functional Genomics, Interfaculty Institute of Genetics and Functional Genomics, University Medicine Greifswald, Greifswald, Germany

[7]Department of Medicine A, University Medicine Greifswald, Greifswald, Germany

[8]Center for Life Course Health Research, University of Oulu, Oulu, Finland

[9]Oulu University Hospital, Oulu, Finland

[10]Department of Epidemiology and Biostatistics, Imperial College London, London, UK

[11]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Universidad del País Vasco (UPV/EHU), San Sebastián, Spain

[12]Primary Health Care Center, Tornio, Finland

[13]Department of Ophthalmology, King's College London, St Thomas' Hospital Campus, London, UK

[14]Unit of Clinical Epidemiology, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden

[15]BioCruces Health Research Institute, Bilbao, Spain

[16]IKERBASQUE, Basque Science Foundation, Bilbao, Spain

Correspondence
Mauro D'Amato, Head, Unit of Gastrointestinal Genetics, Biodonostia HRI, P° Dr. Beguiristain s/n 20014 Donostia-San Sebastián, Gipuzkoa, Spain.
Email: mauro.damato@biodonostia.org

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Abstract

Background: Irritable bowel syndrome (IBS) shows genetic predisposition, however, large-scale, powered gene mapping studies are lacking. We sought to exploit existing genetic (genotype) and epidemiological (questionnaire) data from a series of population-based cohorts for IBS genome-wide association studies (GWAS) and their meta-analysis.

Methods: Based on questionnaire data compatible with Rome III Criteria, we identified a total of 1335 IBS cases and 9768 asymptomatic individuals from 5 independent European genotyped cohorts. Individual GWAS were carried out with sex-adjusted logistic regression under an additive model, followed by meta-analysis using the inverse variance method. Functional annotation of significant results was obtained via

Alexandra Zhernakova and Mauro D'Amato equally contributed.

a computational pipeline exploiting ontology and interaction networks, and tissue-specific and gene set enrichment analyses.

Key Results: Suggestive GWAS signals ($P \leq 5.0 \times 10^{-6}$) were detected for 7 genomic regions, harboring 64 gene candidates to affect IBS risk via functional or expression changes. Functional annotation of this gene set convincingly (best FDR-corrected $P = 3.1 \times 10^{-10}$) highlighted regulation of ion channel activity as the most plausible pathway affecting IBS risk.

Conclusion & Inferences: Our results confirm the feasibility of population-based studies for gene-discovery efforts in IBS, identify risk genes and loci to be prioritized in independent follow-ups, and pinpoint ion channels as important players and potential therapeutic targets warranting further investigation.

KEYWORDS

IBS, SNP, genetics, GWAS, meta-analysis

1 | INTRODUCTION

Irritable bowel syndrome (IBS) affects 10%-15% of people worldwide, is a leading cause of work absenteeism, and consumes 0.5% of the annual healthcare budget.[1] Irritable bowel syndrome is a functional disorder described according to expert consensus (Rome) criteria, based on the presence of symptoms of abdominal pain and bloating associated with constipation (IBS-C), diarrhea (IBS-D), or both (IBS-M).[2,3] The pathophysiology of IBS is poorly understood, although a number of factors have been proposed to contribute including visceral hypersensitivity and altered motility, dysregulated immune activation, miscommunication along the gut-brain axis, bile acid malabsorption, food intolerance, and gut microbiota dysbiosis.[4] In the absence of informative diagnostic tests, increasing hope has been put into genetic studies for the identification of biomarkers and actionable biological pathways that may be exploited for therapeutic purposes.[4]

A heritable component of IBS is long recognized in family and twin studies,[5] and more recently in a nationwide survey of the Swedish population where increased risk was detected among first, second, and third degree relatives of IBS probands.[6] Through candidate gene studies, we have shown that 2%-3% of IBS patients carry rare functional *SI* and *SCN5A* mutations that may link these phenotypes to carbohydrate malabsorption and channelopathies, respectively.[7,8] In addition, associations with common single nucleotide polymorphisms (SNPs) have been reported and replicated for the genes *TNFSF15*, *TRPM8*, *CDC42*, and *NPSR1* (and *SI* and *SCN5A*) by our group and others.[9-14] Yet, IBS gene-hunting efforts have been mostly underpowered to capture modest genetic risk effects, and no unequivocal IBS risk locus has been thus far identified. Despite its described heritability, adequate large-scale hypothesis-free genome-wide association studies (GWAS) have not been reported, possibly because of the lack of suitably sized clinical cohorts with available genetic data.

Key Points

- The pathophysiology of irritable bowel syndrome (IBS) is complex and still poorly understood. A heritable component is long known in IBS but gene-hunting efforts have so far been scarce and no unequivocal IBS risk locus has yet been identified. Here, we report genome-wide association studies (GWAS) and their meta-analysis in 5 population-based cohorts.
- Seven genomic regions, harboring 64 candidate genes were detected. Functional annotation of this gene set highlighted regulation of ion channel activity as the most plausible pathway affecting IBS risk.
- The results support the hypothesis of ion channel involvement in IBS pathophysiology and the feasibility of population-based studies for gene-discovery efforts in IBS.

Because of its high prevalence, we recently hypothesize that a powerful approach to study IBS predisposition may come from the survey of large population-based cohorts, where existing genetic and epidemiological data may be exploited with considerable gain in sample homogeneity and size.[15] Exploiting genotype and questionnaire (Rome Criteria) data available for the TwinGene subset of the Swedish Screening Across Lifespan Twin survey,[16] we recently carried out a pilot population-based GWAS of IBS, where we identified a suggestive signal of association for the KDLR2/GRID2IP region on chromosome 7p22.1 that could be independently replicated in IBS cases and controls from tertiary centers.[17]

Here, we report a meta-analysis of IBS GWAS results from TwinGene and 4 additional population-based cohorts, which

TABLE 1 Demographics of cohorts included in the meta-analysis

Cohort	TOT_{GWAS}[a]	IBS	CTRLS[b]	M:F	Mean age
TwinsUK	2670	180	1041	83:1138	58.4
NFBC1966	4098	353	2499	1314:1538	46
SHIP-TREND	986	59	764	386:437	50.2
TwinGene	9741	522	4810	2531:2801	58.3
LLD	1164	221	654	361:514	48.7
Meta-analysis	18659	1335	9768	4675:6428	53.6

[a]Number of individuals with genotype and phenotype data available.
[b]Asymptomatic subjects.

cumulatively corresponds to a survey of IBS predisposition in 18 659 individuals from 5 Northern European countries (TwinGene from Sweden, LifeLines-DEEP from The Netherlands, SHIP-Trend from Germany, TwinsUK from UK and the Northern Finland Birth Cohort 1966 from Finland; Table 1).

2 | METHODS

2.1 | Study cohorts

Genotype and questionnaire-based epidemiological data from 5 large population cohorts, all previously described in detail, were included in this study. The demographics are reported in Table 1, while a short description of each study cohort and respective criteria for defining IBS cases and controls follows below. All participants provided informed consent, the study protocols were approved by the respective local ethics review boards (see individual cohort information below) and all methods were performed in accordance with the relevant guidelines and regulations.

2.1.1 | TwinGene

TwinGene is a subset of the Screening Across the Lifespan Twin Study initiated in 1998, and focused on twins born in 1958 or before.[16,18] Most of these individuals (n = 45 750, 72.5%) participated in telephone interviews where, among others, GI symptoms were recorded using an adapted version of the Rome criteria module, thoroughly described elsewhere.[19] Genotyping of 9741 individuals from this cohort was performed using Illumina HumanOmniExpress arrays, and TwinGene IBS GWAS results have been previously published.[17] The study was approved by Karolinska Institutet's Ethics Review Board.

2.1.2 | LifeLines-DEEP (LLD)

LifeLines is a large population-based cohort initiated in 2006 in the Netherlands. In 2013, the target size reached over 165 000 participants, aged 25-50 years.[20] LifeLines-DEEP (LLD) is a sub-study of LifeLines where additional biological and health status information has been collected from participants for more in-depth investigations of genetic and epigenetic variation.[21] In particular, questionnaire data on gastrointestinal (GI) health and symptoms, including Rome III Criteria for IBS are available for LLD. Harmonized genotype data (Illumina HumanCytoSNP-12 BeadChip and Illumina ImmunoChip) were available for 1164 participants. The Ethics Committee at the University Medical Centre Groningen approved the study.

2.1.3 | SHIP-Trend

The Study of Health in Pomerania (SHIP)-Trend is a cross-sectional, population-based survey in North-Eastern Germany including 3086 participants (49.4% men; age 20-82 years).[22] Enrollment started in 2008, was continued until 2012, and the first follow-up investigations began in 2015. Questionnaire data collected for this cohort included information compatible with the Rome III Criteria for IBS, which were used to classify individuals into IBS cases and asymptomatic controls. Illumina HumanOmni 2.5M genotype data were available for 986 participants. Study and protocol approval were obtained from the Institutional Review Board of the University of Greifswald in Germany.

2.1.4 | TwinsUK

This cohort based at St. Thomas' Hospital in London is a volunteer cohort of over 10 000 twins from the general population, aged 18-103 years (in 2013).[23] Harmonized genotype data (Illumina HumanHap300 BeadChip and Illumina HumanHap610 QuadChip) were accessible for 2670 subjects with IBS phenotypic information available. Phenotypic status (IBS case or asymptomatic control) was defined based on GI symptoms from questionnaire data compatible with the Rome III Criteria. Participants gave fully informed consent under a protocol reviewed by the St. Thomas' Hospital Local Research Ethics Committee.

2.1.5 | Nfbc1966

The Northern Finland Birth Cohort 1966 (NFBC1966) is a population-based birth cohort with a collection of data from 96.3% (N = 12 058) of all people born in the northern part of Finland in the year 1966.[24] The follow-up questionnaire from 2012, when participants were 45-46 years old, contains questions on GI symptoms compatible with the Rome III criteria. Genotype data (Illumina Infinium CNV 370-Duo)

were available for 4098 NFBC1966 participants, and were used in this study. NFBC1966 participants gave their informed consent and the Regional Ethics Committee of the Northern Ostrobothnia District approved the study.

2.2 | Genotype quality control (QC) and individual GWA studies

Genotype and phenotype data from 875 LLD individuals (221 IBS cases and 654 controls), 5332 TwinGene individuals (522 IBS cases and 4810 controls), 823 SHIP-Trend individuals (59 IBS cases and 764 controls), 1221 TwinsUK individuals (180 IBS cases and 1041 controls), and 2852 NFBC1966 individuals (353 IBS cases and 2499 controls) were included in the analyses. Genome-wide association studies association tests were performed after standard QC procedures: SNPs with genotype call rate <98% and/or a Hardy-Weinberg equilibrium (HWE) $P < 1.0 \times 10^{-7}$ were excluded, together with individuals with more than 2% missing genotypes and/or pairwise relatedness score >0.15. Imputation with IMPUTE2 and the 2.5 million HapMap (CEU rel22) SNP panel reference was performed separately on individual datasets at Karolinska Institutet (for TwinGene and SHIP-Trend) or at respective local computing facilities (for LLD, TwinsUK, NFBC1966). The absence of population stratification was tested for each cohort through principal component analyses and any ancestry other than European was excluded from the regression models. Individual case-control association tests were performed using regression methods implemented in PLINK (TwinGene, SHIP-TREND, TwinsUK) and SNPTEST (LLD, NFBC1966).[25,26]

2.3 | GWAS meta-analysis

Prior to meta-analysis, individual GWAS association results was checked by using the R package EasyQC (v9.2) in order to identify study-specific problems, remove missing/invalid data or duplicates, and harmonize allele coding among datasets.[27] The absence of population stratification was controlled for based on the genomic control inflation factor ($\lambda < 1.10$ for all cohorts). All GWAS results were filtered for SNP markers with minor allele frequency (MAF) >0.01 and imputation quality score (INFO > 0.8) before meta-analysis. A total of 2 483 385 high-quality SNP markers passing QC, with available summary statistics from at least 2 datasets, and the absence of heterogeneity across studies (Cochran's Q > 0.05) were brought forward into the meta-analysis. Meta-analysis was performed with the statistical software METAL using the fixed-effect method weighted by inverse variance.[28]

Manhattan plots were generated with the R package GWASTools,[29] while LocusZoom (locuszoom.org)[30] was used to individually plot meta-analysis association signals for suggestive IBS risk loci. Meta-analysis results were also explored in relation to previously reported IBS gene associations, and SNPs mapping within a 2 kb window upstream/downstream of the corresponding coding regions.

2.4 | Post-GWAS functional genomics analyses

The IBS risk loci were defined as non-overlapping genomic regions extending ±250 Kb from (suggestive) association signals with $P \leq 5.0 \times 10^{-6}$. Annotation of genes mapping to the identified risk loci was performed based on risk loci genomic coordinates using the XGR package.[31] The IBS risk loci were examined for quantitative cis-effects over gene expression levels (eQTLs) by screening the publicly available Genotype-Tissue Expression (GTEx) database containing precomputed eQTL data for ~19.5M significant associations between SNP markers and 44 human tissues (version v6).[32] A final list totaling 64 annotated IDs of genes mapping within (GWAS genes) and/or in the flanking regions (SNP-eQTL genes) of IBS suggestive risk loci was built, and used for preliminary biological interpretation of GWAS findings using computational approaches to functional characterizations. Tissue-specific enrichment analysis was performed with the Tissue Specific Expression Analysis (TSEA) tool (http://genetics.wustl.edu/jdlab/tsea/) using Fischer's exact test and Benjamini-Hochberg correction to detect significant overrepresentation of IBS risk genes among transcripts from individual tissues.[33] Functional annotation via gene set enrichment analysis (GSEA) was obtained with functional class scoring against Gene Ontology (GO) terms (molecular function and biological process) and the Molecular Signatures Database (MsigDB module C4).[34] Hypergeometric tests and false discovery rate (FDR) correction (FDR-corrected $P \leq 0.05$) were used together with XGR default settings to test for enrichment.

3 | RESULTS

3.1 | Computational strategy

We implemented a bioinformatic pipeline for a step-wise analysis of collected data, foreseeing (1) data quality control (QC) and single GWAS from individual cohorts, (2) harmonization and meta-analysis of GWAS results, and (3) functional annotation of the identified risk regions in order to gain biological insight from the observed associations. A schematic summary of this strategy is reported in Table 2, together with the computational tools and protocols adopted for the respective analyses.

3.2 | GWAS meta-analysis

Based on questionnaire data from genotyped participants in individual cohorts, IBS prevalence ranged from 5.4% (TwinGene) to 19.0% (LifeLines-DEEP) in the 5 datasets. In addition to the already published TwinGene study,[17] independent GWASs were carried out in the 4 additional cohorts using sex-adjusted logistic regression under an additive model. Respective GWA studies did not yield significant ($P \leq 5.0 \times 10^{-8}$) genome-wide association findings in individual cohorts (not shown). Harmonization and QC filtering of combined datasets allowed GWAS association results from a total of 1335 IBS cases and 9768 asymptomatic controls to be included in a meta-analysis harnessing 2 483 358 high-quality SNP markers. Although

TABLE 2 Summary of the computational strategy

Phase	Step	Description	Tool	Links
Quality control and GWAS of individual datasets	GENOTYPE QC	SNP call rate>0.95; MAF>0.01; INFO> 0.8; P-HWE>1.0E-07	PLINK; SNPTEST	https://www.cog-genomics.org/plink2; https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html
	SAMPLE QC	No PCA outlier; call rate>0.98; pairwise relatedness>0.15	PLINK; SNPTEST	https://www.cog-genomics.org/plink2; https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html
	IMPUTATION	Inference of unobserved genotypes using reference panels	IMPUTE2	http://mathgen.stats.ox.ac.uk/impute/impute_v2.html
	ASSOCIATION	Sex-adjusted logistic regression	PLINK; SNPTEST	https://www.cog-genomics.org/plink2; https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html
Meta-analysis	DATASET QC	No invalid/missing data; harmonized allele coding; aligned to 1KG reference panel	EasyQC	http://www.uni-regensburg.de/medizin/epidemiologie-praeventivmedizin/genetische-epidemiologie/software/index.html
	META-ANALYSIS	Inverse variance weighted method	METAL	https://genome.sph.umich.edu/wiki/METAL
Functional Annotation	DEFINING RISK LOCI	Non-overlapping genomic regions extending ±250 Kb from association signals with $P \leq 5.0E-06$	R	https://www.r-project.org/
	GENE MAPPING	Positional and eQTL genes mapping	XGR; GTEx	http://galahad.well.ox.ac.uk:3020/; https://www.gtexportal.org/
	FUNCTIONAL CLASS SCORING	Gene set enrichment analysis using Gene Ontology and Molecular Signature Database	XGR	http://galahad.well.ox.ac.uk:3020/
	TISSUE ENRICHMENT ANALYSIS	Test overrepresentation of risk genes in transcription from individual tissues	TSEA	http://genetics.wustl.edu/jdlab/tsea/

no SNP reached genome-wide significance (Figure 1), 7 independent loci gave rise to suggestive ($P \leq 5.0 \times 10^{-6}$) association signals, which also showed concordance of genetic risk effects in individual cohorts (Table 3). The strongest association signal was obtained for the marker rs17112758 on chromosome 1 ($P = 1.89 \times 10^{-6}$), in a region where the phospholipid phosphatase 3 (*PLPP3*) gene maps.

3.3 | Downstream analyses of suggestive IBS risk loci

Based on a computational annotation of gene content and gene expression from respective regions (see Methods and Table 2), IBS risk loci were predicted to harbor 35 genes and several SNPs affecting the expression of 29 additional nearby genes (expression quantitative trait loci—eQTL; Table 3), thus bringing the total number of transcripts of interest to 64.

Tissue-specific enrichment analysis (TSEA) of expression in different human tissues indicated nominally significant higher expression of these genes in the colon compared to other sites ($P = 0.027$). An ontology-based GSEA using the risk gene set for functional class scoring (see Methods and Table 2) returned strongly significant results for ion channel activities both at the level of molecular function (Gene Ontology terms *sodium channel regulator activity* [GO:0017080] FDR-corrected $P = 1.2 \times 10^{-7}$; *channel regulatory activity* [GO:0016247] FDR-corrected $P = 8.5 \times 10^{-6}$; *ion channel activity* [GO:0005216] FDR-corrected $P = 6.7 \times 10^{-3}$), and biological process (*regulation of sodium ion transmembrane transporter activity* [GO:2000649], FDR-corrected $P = 3.1 \times 10^{-10}$; *regulation of*

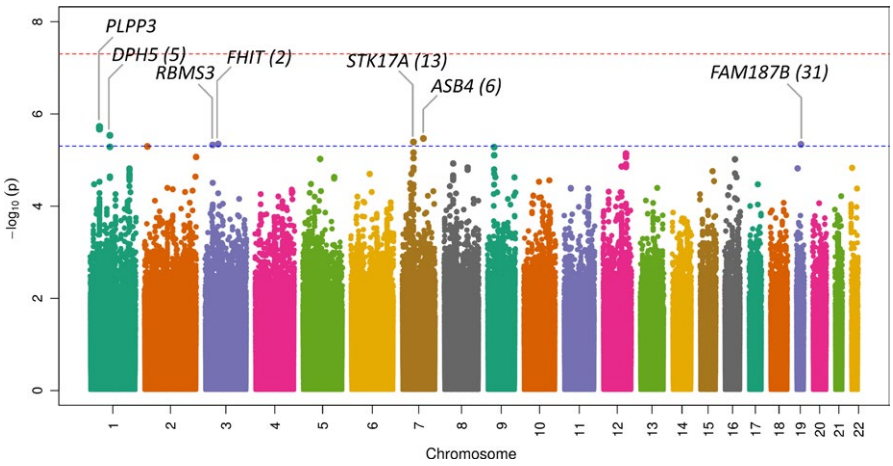

FIGURE 1 Manhattan plot of IBS GWAS meta-analysis results. Genome-wide ($P = 5 \times 10^{-8}$) and suggestive ($P = 5 \times 10^{-6}$) significance levels are indicated, respectively, with red and blue horizontal dashed lines. For each suggestive association signal, the nearest gene (mapping closest to the lead SNP) is reported, together with the number of additional genes from the same locus (in brackets)

TABLE 3 GWAS meta-analysis results

Lead SNP	CHR	EA	EAF	*P*	OR	Genomic content[a]
rs17112758 (A/G)	1	A	0.174	1.89E-06	1.29	*PLPP3*
rs72987295 (A/G)	1	A	0.246	2.93E-06	1.67	*DPH5*, *S1PR1*, *SLC30A7*, *EXTL2*, *GPR88*, *CDC14A*
rs7427882 (A/C)	3	A	0.248	4.70E-06	0.77	*RBMS3*
rs2366846 (A/C)	3	A	0.177	4.52E-06	1.40	*FHIT*, *FAM3D*, *KCTD6*
rs7802995 (G/A)	7	G	0.226	4.09E-06	0.72	*HECW1*, *LOC100506895*, *BLVRA*, *COA1*, *STK17A*, *AEBP1*, *POLM*, *URGCP*, *GCK*, *DBNL*, *UBE2D4*, *SPDYE1*, *C7orf25*, *POLD2*
rs6973126 (C/T)	7	C	0.486	3.41E-06	1.22	*ASB4*, *PDK4*, *PON1*, *PON2*, *PON3*, *PPP1R9A*, *DYNC1I1*
rs10424110 (G/A)	19	G	0.273	4.58E-06	1.66	*HAMP*, *HPN-AS1*, *MIR5196*, *CD22*, *FAM187B*, *FFAR1*, *FFAR2*, *FFAR3*, *FXYD1*, *FXYD3*, *FXYD5*, *FXYD7*, *GRAMD1A*, *HPN*, *LGI4*, *LSR*, *MAG*, *SCN1B*, *USF2*, *GPR42*, *ZNF30*, *SDHAF1*, *TYROBP*, *COX6B1*, *CAPNS1*, *HSPB6*, *KMT2B*, *ZNF792*, *AC020907.1*, *UPK1A*, *U2AF1L4*, *DMKN*

CHR: chromosome; EA: effect allele; EAF: effect allele frequency.

[a]Genes from individual risk loci (250 kb either side of lead SNP); both genes physically mapping to the region and genes associated with eQTLs (underlined) are reported.

transporter activity [GO:0032409] FDR-corrected $P = 8.9 \times 10^{-6}$) (Figure 2). In addition, an alternative functional classification using curated gene sets from the Molecular Signatures Database (Methods and Table 2),[34] likewise provided strong evidence of enrichment for the *Ion channels* category, together with *Liver genes—metabolism and xenobiotics* (FDR-corrected $P = 0.0017$ for both).

3.4 | IBS risk genes from previous studies

Meta-analysis results were also inspected specifically at loci previously proposed to affect risk of IBS and/or its intermediate phenotypes—colonic transit, abdominal pain, and sensory ratings.[15] Table 4 reports meta-analysis summary statistics corresponding

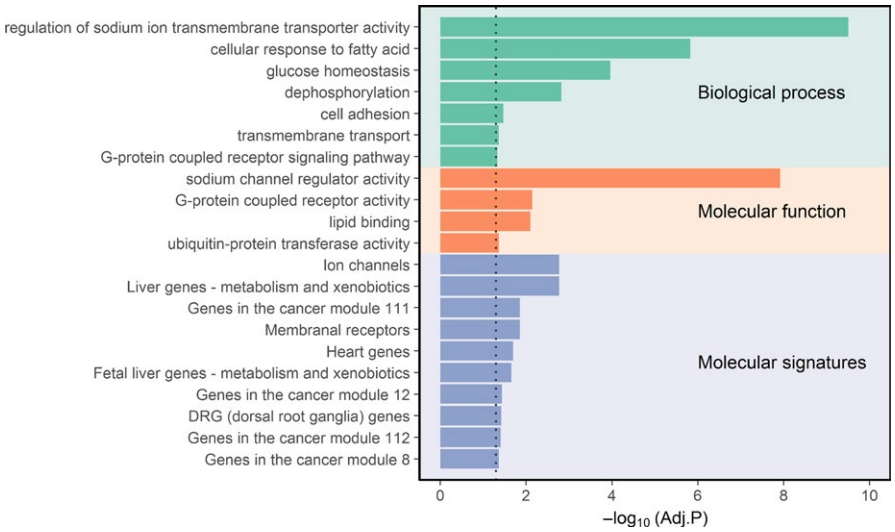

FIGURE 2 Gene set enrichment analysis results. Top Gene Ontology terms (Molecular Function and Biological Process) and Molecular Signature Database sets are ranked by adjusted *P*-value after FDR correction (the dotted line indicates cut-off significance at $P = 0.05$)

TABLE 4 GWAS meta-analysis results for previously reported IBS risk genes

SNP[a]	CHR	EA	EAF	P[a]	OR	N SNPs in region	N SNPs with P < 0.05	Gene
rs2473322 (T/G)	1	T	0.08	**3.60E-02**	0.85	35	17	*CDC42*
rs3024491 (C/A)	1	A	0.47	1.10E-01	0.9	21	0	*IL10*
rs758277 (C/T)	2	T	0.08	**2.50E-03**	0.86	224	53	*TRPM8*
rs7430407 (C/T)	3	T	0.12	**5.40E-04**	0.72	104	9	*SCN5A*
rs187084 (A/G)	3	A	0.42	5.30E-01	0.97	3	0	*TLR9*
rs75982521 (C/T)	3	T	0.13	**2.60E-03**	1.52	76	4	*SI*
rs12634774 (C/T)	3	T	0.41	4.70E-01	0.96	8	0	*HTR3E*
rs13146907 (A/G)	4	A	0.36	**1.20E-02**	1.19	50	6	*KLB*
rs2243270 (A/G)	5	A	0.23	1.30E-01	1.07	17	0	*IL4*
rs3093671 (G/A)	6	A	0.02	**3.10E-02**	1.51	14	1	*TNF*
rs10499050 (A/G)	6	A	0.04	1.30E-01	0.83	29	0	*PRDM1*
rs4574760 (C/T)	7	T	0.37	**4.00E-04**	1.17	163	7	*KDELR2/ GRID2IP*
rs10441147 (T/C)	7	T	0.29	**6.60E-03**	0.84	521	95	*NXPH1*
rs324381 (G/A)	7	A	0.35	**1.30E-03**	0.86	323	46	*NPSR1*
rs7867918 (G/T)	9	T	0.13	**3.50E-02**	1.2	18	1	*TNFSF15*
rs4963516 (T/G)	12	T	0.17	**6.20E-03**	0.85	12	1	*GNB3*
rs12597188 (G/A)	16	A	0.34	**1.90E-02**	0.9	100	4	*CDH1*
rs8071583 (A/T)	17	A	0.03	1.40E-01	1.49	26	0	*SLC6A4*

CHR: chromosome; EA: effect allele; EAF: effect allele frequency.

[a]Best association signal (lowest *P*-value) from the region containing the coding sequence + 2 kb each side. Significant associations ($P \leq 0.05$) are highlighted in bold.

to genes and loci selected based on prior IBS associations from existing literature. We observed nominal association for at least one SNP marker in 12 of 18 inspected loci, with best evidence of replication for the genes *SCN5A*, *SI*, *NPSR1*, and *TRPM8*, as well the 7p22.1 *KDELR2/GRID2IP* region from our previous IBS GWAS (Table 4).

4 | DISCUSSION

We report here a GWAS meta-analysis of 5 IBS studies from general population-based cohorts totaling 18 659 individuals, and including 1335 cases and 9768 asymptomatic controls. We report suggestive evidence ($P \leq 5.0 \times 10^{-6}$) for 7 IBS risk loci, which correspond to 64 candidate risk genes either physically mapping to the risk loci or showing expression affected by SNPs contained within these risk loci (eQTL genes). While respective association signals are largely comparable (almost identical *P*-values), stronger genetic risk effects appear to be imparted by risk loci on chromosome 1 and chromosome 19 (ORs 1.67 and 1.66, respectively). Individual causative genes cannot be singled out at this stage, although it is interesting to note that these 2 regions harbor, respectively, the G protein-coupled receptor 88 (*GPR88*) playing a role in the regulation of cognitive and motor function, and a cluster of ion channel genes including *SCN1B* and the *FXYD* family, which are involved in the control of smooth muscle excitability (discussed more in detail below).

Our downstream functional (GSEA) analysis of the suggestive GWAS loci yielded statistically sound, compelling evidence that the corresponding IBS risk gene pool is enriched for transcripts involved in the regulation of ion channel activity (*sodium channel regulator activity* and *regulation of sodium ion transmembrane transporter activity* were the highly significant, top-ranking GO terms for the classes *molecular function* and *biological process*, respectively). This finding is noteworthy, as it replicates and reinforces our previous observations that DNA variations affecting ion channel function(s) result in detectable genetic risk effects in IBS and its intermediate phenotypes.[7,11,35] Indeed, rare variants and common SNPs affecting IBS risk have been identified in the ion channel genes *SCN5A* (coding for the voltage-gated sodium channel NaV1.5 associated with various forms of arrhythmia including Brugada syndrome)[36] and *TRPM8* (coding for the "cold-" and menthol-receptor transient receptor potential cation channel subunit M member 8),[11] while recent GWAS results highlighted *ion channel activity* and *xenobiotic metabolism* among the biological pathways most relevant to stool frequency (*metabolism and xenobiotics* being a molecular signature identified also here through GSEA downstream analysis of GWAS results).[35]

Ion channels are located in the membranes of all types of cells and coordinate ion passage across membranes in response to different stimuli.[37] They have also cell-specific functions and, particularly in the gut, they play important roles in GI functions relevant to IBS, such as secretion and absorption of electrolytes and fluids, hormone secretion, control of motility and visceral sensation through neuronal signaling, and induction of smooth muscle contraction.[38-41] Dysfunctional ion channels can lead to conditions known as channelopathies, which indeed most often develop because of deleterious mutations in the corresponding genes. Channelopathies are well known in the fields of cardiology and neurology, contributing to several complex conditions including cardiac arrhythmias and pain-related neuropathies.[42-44] In these conditions, patients often report bowel complaints, and this has led to the hypothesis that specific ion channels may also be relevant to IBS.[45,46] Hypomorphic *SCN5A* variants have been identified, possibly affecting IBS risk due to their reduced NaV1.5 smooth muscle pacemaker activity on the interstitial Cells of Cajal (ICC).[7,47,48] Interestingly, as mentioned, one of the genes mapping to the suggestive risk locus on chromosome 19 identified here, *SCN1B* (Table 3), encodes a β subunit of the NaV1.5 sodium channel and, like *SCN5A*, has been implicated in Brugada syndrome and other cardiac arrhythmia syndromes,[36,49] hence deserves attention in follow-up studies as it might represent the best causative candidate gene from that region. However, also from the chromosome 19 locus are other ion channel-related genes *FXYD1*, *FXYD3*, *FXYD5*, *and FXYD7*, whose expression appears to be affected

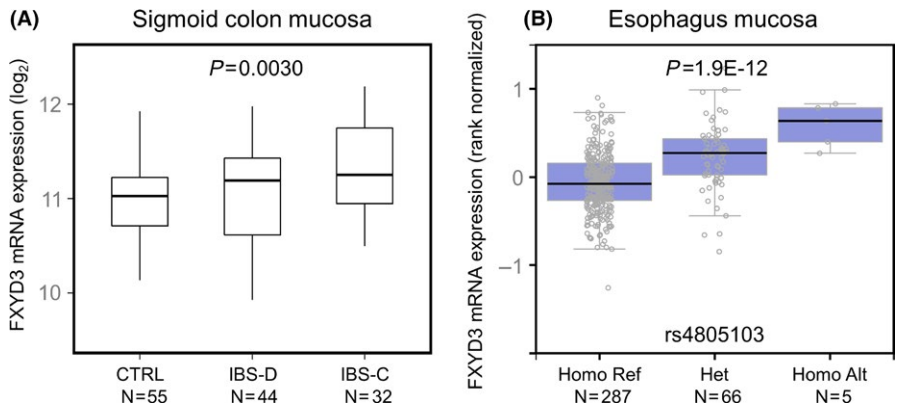

FIGURE 3 *FXYD3* publicly available gene expression data. A: Comparison of *FXYD3* sigmoid colon mRNA levels in IBS patients and controls, as from the TIBS database (http://www.chengfeng.info/tibs_database.html). B: Example of *FXYD3* locus-specific SNP-eQTL effects: shown is the correlation between SNP rs4805103 genotype and esophagus mucosa *FXYD3* mRNA expression, as from data publicly available at the GTEx project portal (www.gtexportal.org)

by genetic variation in the region (via eQTLs). These code for small membrane proteins, known as FXYD(Phe-x-Tyr-Asp)-domain containing ion transport regulators, which interact with ion transporters such as the Na^+/K^+-ATPase and often modulate tissue-specific functions and excitability.[50] In addition, *FXYD5* (dysadherin) expression appears to affect intestinal cell-cell adhesion via dilation of tight and adherent junction proteins including E-cadherin.[51,52] Also of interest, gene expression data publicly available from TIBS[53] and GTEx[32] databases show, respectively, higher sigmoid colon *FXYD3* mRNA expression in IBS patients compared to controls, and strong SNP-eQTL effects from the *FXYD3* locus in the esophageal mucosa (reported in Figure 3). Genotype-driven changes in the expression of *FXYD3* or other FXYD genes may, therefore, ultimately affect intestinal permeability and epithelial barrier integrity, with downstream effects on mucosal responses to enteric bacteria and luminal compounds, immune activation, visceral sensation, and other bowel functions of potential relevance to IBS predisposition.[54,55]

Altogether, several lines of evidence coming from different studies and approaches support the involvement of ion channels and related genes in the pathophysiology of IBS. This may be important for future therapeutic exploitation, as these channels are attractive drug targets most often accessible on the cell surface, thus representing ideal gateways to actionable pathways.[39,45,56] Two examples already exist of such potential exploitation: the identification of *SCN5A* mutations in IBS has led to the successful proof-of-principle administration of mexiletine (antiarrhythmic drug) to normalize stool frequency in a severely constipated *SCN5A*-carrier patient;[7] the reported associations of the menthol-receptor gene *TRPM8* with constipation-predominant IBS and harder stools in the general population[11] open up, for instance, for the (re-)evaluation of peppermint oil and/or Iberogast® IBS trials, as the efficacy of these treatments in reducing bowel symptoms may be better assessed upon patients' stratification according to *TRPM8* genotype.

Our meta-analysis results were also investigated in relation to previously reported IBS candidate risk genes (Table 4), and nominal replication ($P \leq 0.05$) was obtained for 12 of 18 tested loci. While borderline associations may be more difficult to properly evaluate, best association signals (lowest *P*-values) were detected for the *KDELR2/GRID2IP* locus on chromosome 7 and the genes *TRPM8*, *SCN5A*, *NPSR1*, and *SI* (Table 4). This result may be expected for the first 3 loci, which were already detected in the original TwinGene GWAS[17] that contributes 43% of the variance to the current meta-analysis, although *NPSR1* and *SI* signals may represent *bona fide* replications. *NPSR1* (neuropeptide S receptor gene) is a neuropeptide receptor involved in anxiety, inflammation, and nociception, and we previously reported its association with colonic transit and sensory ratings in IBS patients,[13] and recurrent abdominal pain in Swedish children from a population-based birth cohort.[14] The *SI* gene codes for sucrase-isomaltase, an intestinal disaccharidase that digests 60% of dietary starch and sucrose and is mutated in patients suffering from congenital sucrase-isomaltase deficiency (CSID, also called sucrose intolerance). This condition is due to colonic accumulation of undigested carbohydrates that provoke symptoms largely overlapping with those observed in IBS patients (CSID is also sometimes misdiagnosed as IBS), and we recently demonstrated that functional *SI* variants with reduced SI enzymatic activity associate with increased risk of IBS, particularly IBS-D.[8] Replicating *SI* associations with IBS in a population-based sample is, therefore, a noteworthy finding from this study. Weaker replication signals, for instance in the IBS risk (immune-related) gene *TNFSF15* consistently replicated in independent case-control studies after our original identification,[9,10,12] may be due to dilution of specific (immune-mediated) genetic risk effects when assessing associations with IBS solely defined based on questionnaire data.

In conclusion, we report a meta-analysis of 5 independent IBS GWA studies including a total of 1335 patients and 9768 asymptomatic controls. Its outcome demonstrates the feasibility of population-based approaches to gene discovery in IBS, identifies 7 candidate risk loci to prioritize in follow-up validation efforts, and highlights the biology of ion channels as a plausible IBS pathophysiological pathway that warrants further investigation.

COMPETING INTERESTS

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AUTHOR CONTRIBUTIONS

MDA and AZ study concept and design; FW, PH, CW, JRO, LB, and TK, data acquisition; FB, MH, TZ, WEE, NVR, MM, FH, GH, and VK statistical analyses; MH, FB, MDA, VK, AR, AN, MCC, ET, AAA, and JRA data analysis and interpretation; MDA obtained funding, administrative and technical support, and study supervision; FB, MH, and MDA drafted the manuscript, with input and critical revision from all other authors. All authors approved the final draft of the manuscript.

ORCID

F. Bonfiglio http://orcid.org/0000-0003-2488-3867

M. D'Amato http://orcid.org/0000-0003-2743-5197

REFERENCES

1. Canavan C, West J, Card T. The epidemiology of irritable bowel syndrome. *Clin Epidemiol*. 2014;6:71-80.
2. Drossman DA. The functional gastrointestinal disorders and the Rome III process. *Gastroenterology*. 2006;130:1377-1390.
3. Drossman DA, Hasler WL. Rome IV—Functional GI disorders: disorders of gut-brain interaction. *Gastroenterology*. 2016;150:1257-1261.
4. Enck P, Aziz Q, Barbara G, et al. Irritable bowel syndrome. *Nat Rev Dis Prim*. 2016;2:16014.
5. Saito YA. The role of genetics in IBS. *Gastroenterol Clin North Am*. 2011;40:45-67.
6. Waehrens R, Ohlsson H, Sundquist J, et al. Risk of irritable bowel syndrome in first-degree, second-degree and third-degree relatives of affected individuals: a nationwide family study in Sweden. *Gut*. 2015;64:215-221.

7. Beyder A, Mazzone A, Strege PR, et al. Loss-of-function of the voltage-gated sodium channel NaV1.5 (channelopathies) in patients with irritable bowel syndrome. *Gastroenterology*. 2014;146:1659-1668.
8. Henström M, Diekmann L, Bonfiglio F, et al. Functional variants in the sucrase-isomaltase gene associate with increased risk of irritable bowel syndrome. *Gut*. 2018;67:263-270.
9. Zucchelli M, Camilleri M, Andreasson AN, et al. Association of TNFSF15 polymorphism with irritable bowel syndrome. *Gut*. 2011;60:1671-1677.
10. Swan C, Duroudier NP, Campbell E, et al. Identifying and testing candidate genetic polymorphisms in the irritable bowel syndrome (IBS): association with TNFSF15 and TNFα. *Gut*. 2013;62:985-994.
11. Henström M, Hadizadeh F, Beyder A, et al. TRPM8 polymorphisms associated with increased risk of IBS-C and IBS-M. *Gut*. 2016;66:1725-1727.
12. 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*. 2014;63:1103-1111.
13. Camilleri M, Carlson P, Zinsmeister AR, et al. Neuropeptide S receptor induces neuropeptide expression and associates with intermediate phenotypes of functional gastrointestinal disorders. *Gastroenterology*. 2010;138(98–107):e4.
14. Henström M, Zucchelli M, Söderhäll C, et al. NPSR1 polymorphisms influence recurrent abdominal pain in children: a population-based study. *Neurogastroenterol Motil*. 2014;26:1417-1425.
15. D'Amato M. Genes and functional GI disorders: from casual to causal relationship. *Neurogastroenterol Motil*. 2013;25:638-649.
16. Lichtenstein P, Sullivan PF, Cnattingius S, et al. The Swedish Twin Registry in the third millennium: an update. *Twin Res Hum Genet*. 2006;9:875-882.
17. 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*. 2015;64:1774-1782.
18. Lichtenstein P, De Faire U, Floderus B, et al. The Swedish Twin Registry: a unique resource for clinical, epidemiological and genetic studies. *J Intern Med*. 2002;252:184-205.
19. Svedberg P, Johansson S, Wallander M-A, et al. No evidence of sex differences in heritability of irritable bowel syndrome in Swedish twins. *Twin Res Hum Genet*. 2008;11:197-203.
20. Scholtens S, Smidt N, Swertz MA, et al. Cohort Profile: LifeLines, a three-generation cohort study and biobank. *Int J Epidemiol*. 2015;44:1172-1180.
21. Tigchelaar EF, Zhernakova A, Dekens JAM, et al. Cohort profile: LifeLines DEEP, a prospective, general population cohort study in the northern Netherlands: study design and baseline characteristics. *BMJ Open*. 2015;5:e006772.
22. Völzke H, Alte D, Schmidt CO, et al. Cohort profile: the study of health in Pomerania. *Int J Epidemiol*. 2011;40:294-307.
23. Moayyeri A, Hammond CJ, Hart DJ, et al. The UK Adult Twin Registry (TwinsUK Resource). *Twin Res Hum Genet*. 2013;16:144-149.
24. Rantakallio P. The longitudinal study of the Northern Finland birth cohort of 1966. *Paediatr Perinat Epidemiol*. 1988;2:59-88.
25. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559-575.
26. Marchini J, Howie B, Myers S, et al. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet*. 2007;39:906-913.
27. Winkler TW, Day FR, Croteau-Chonka DC, et al. Quality control and conduct of genome-wide association meta-analyses. *Nat Protoc*. 2014;9:1192-1212.
28. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*. 2010;26:2190-2191.
29. Gogarten SM, Bhangale T, Conomos MP, et al. GWASTools: an R/Bioconductor package for quality control and analysis of genome-wide association studies. *Bioinformatics*. 2012;28:3329-3331.
30. Pruim RJ, Welch RP, Sanna S, et al. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics*. 2010;26:2336-2337.
31. Fang H, Knezevic B, Burnham KL, et al. XGR software for enhanced interpretation of genomic summary data, illustrated by application to immunological traits. *Genome Med*. 2016;8:129.
32. Ardlie KG, Deluca DS, Segre AV, et al. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science*. 2015;348:648-660.
33. Dougherty JD, Schmidt EF, Nakajima M, et al. Analytical approaches to RNA profiling data for the identification of genes enriched in specific cells. *Nucleic Acids Res*. 2010;38:4218-4230.
34. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA*. 2005;102:15545-15550.
35. Jankipersadsing SA, Hadizadeh F, Bonder MJ, et al. A GWAS meta-analysis suggests roles for xenobiotic metabolism and ion channel activity in the biology of stool frequency. *Gut*. 2017;66:756-758.
36. Sarquella-Brugada G, Campuzano O, Arbelo E, et al. Brugada syndrome: clinical and genetic findings. *Genet Med*. 2016;18:3-12.
37. Ashcroft F. *Ion Channels and Disease*. Burlington: Elsevier Science; 2000.
38. Fuentes IM, Christianson JA. Ion channels, ion channel receptors, and visceral hypersensitivity in irritable bowel syndrome. *Neurogastroenterol Motil*. 2016;28:1613-1618.
39. Blackshaw LA, Brierley SM, Hughes PA. TRP channels: new targets for visceral pain. *Gut*. 2010;59:126-135.
40. Blackshaw LA. Transient receptor potential cation channels in visceral sensory pathways. *Br J Pharmacol*. 2014;171:2528-2536.
41. Holzer P. Acid-sensitive ion channels and receptors. *Handb Exp Pharmacol*. 2009;283-332.
42. Julius D. TRP channels and pain. *Annu Rev Cell Dev Biol*. 2013;29:355-384.
43. Spillane J, Kullmann DM, Hanna MG. Genetic neurological channelopathies: molecular genetics and clinical phenotypes. *J Neurol Neurosurg Psychiatry*. 2016;87:37-48.
44. Campuzano O, Beltrán-Álvarez P, Iglesias A, et al. Genetics and cardiac channelopathies. *Genet Med*. 2010;12:260-267.
45. Beyder A, Farrugia G. Targeting ion channels for the treatment of gastrointestinal motility disorders. *Therap Adv Gastroenterol*. 2012;5:5-21.
46. Beyder A, Farrugia G. Ion channelopathies in functional GI disorders. *Am J Physiol Gastrointest Liver Physiol*. 2016;311:G581-G586.
47. Ou Y, Gibbons SJ, Miller SM, et al. SCN5A is expressed in human jejunal circular smooth muscle cells. *Neurogastroenterol Motil*. 2002;14:477-486.
48. Strege PR, Ou Y, Sha L, et al. Sodium current in human intestinal interstitial cells of Cajal. *Am J Physiol Gastrointest Liver Physiol*. 2003;285:G1111-G1121.
49. Watanabe H, Koopmann TT, Le Scouarnec S, et al. Sodium channel β1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. *J Clin Invest*. 2008;118:2260-2268.
50. Crambert G, Geering K. FXYD proteins: new tissue-specific regulators of the ubiquitous Na,K-ATPase. *Sci STKE*. 2003;2003:RE1.
51. Lubarski I, Pihakaski-Maunsbach K, Karlish SJD, et al. Interaction with the Na, K-ATPase and tissue distribution of FXYD5 (related to ion channel). *J Biol Chem*. 2005;280:37717-37724.

52. Lubarski Gotliv I. FXYD5: Na(+)/K(+)-ATPase regulator in health and disease. *Front Cell Dev Biol*. 2016;4:26.
53. Yan J, Xu Y, Hu B, et al. TIBS: a web database to browse gene expression in irritable bowel syndrome. *J Theor Biol*. 2014;354:48-53.
54. Barbara G, Wang B, Stanghellini V, et al. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. *Gastroenterology*. 2007;132:26-37.
55. Piche T. Tight junctions and IBS–the link between epithelial permeability, low-grade inflammation, and symptom generation? *Neurogastroenterol Motil*. 2014;26:296-302.
56. Bagal SK, Brown AD, Cox PJ, et al. Ion channels as therapeutic targets: a drug discovery perspective. *J Med Chem*. 2013;56:593-624.

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