



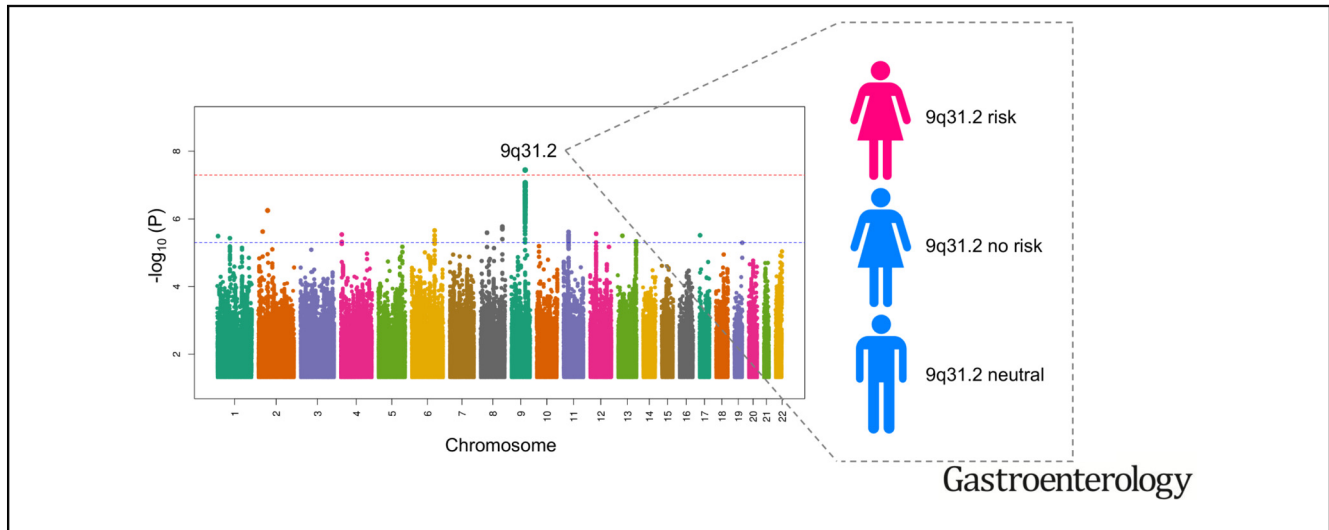
# Female-Specific Association Between Variants on Chromosome 9 and Self-Reported Diagnosis of Irritable Bowel Syndrome

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**BACKGROUND & AIMS:** Genetic factors are believed to affect risk for irritable bowel syndrome (IBS), but there have been no sufficiently powered and adequately sized studies. To identify DNA variants associated with IBS risk, we performed a genome-wide association study (GWAS) of the large UK Biobank population-based cohort, which includes genotype and health data from 500,000 participants. **METHODS:** We studied 7,287,191 high-quality single nucleotide polymorphisms in individuals who self-reported a doctor's diagnosis of IBS (cases; n = 9576) compared to the remainder of the cohort (controls; n = 336,499) (mean age of study subjects, 40–69 years). Genome-wide significant findings were further investigated in

2045 patients with IBS from tertiary centers and 7955 population controls from Europe and the United States, and a small general population sample from Sweden (n = 249). Functional annotation of GWAS results was carried out by integrating data from multiple biorepositories to obtain biological insights from the observed associations. **RESULTS:** We identified a genome-wide significant association on chromosome 9q31.2 (single nucleotide polymorphism rs10512344;  $P = 3.57 \times 10^{-8}$ ) in a region previously linked to age at menarche, and 13 additional loci of suggestive significance ( $P < 5.0 \times 10^{-6}$ ). Sex-stratified analyses revealed that the variants at 9q31.2 affect risk of IBS in women only ( $P = 4.29 \times 10^{-10}$  in UK Biobank) and also



associate with constipation-predominant IBS in women ( $P = .015$  in the tertiary cohort) and harder stools in women ( $P = .0012$  in the population-based sample). Functional annotation of the 9q31.2 locus identified 8 candidate genes, including the elongator complex protein 1 gene (*ELP1* or *IKBKAP*), which is mutated in patients with familial dysautonomia. **CONCLUSIONS:** In a sufficiently powered GWAS of IBS, we associated variants at the locus 9q31.2 with risk of IBS in women. This observation may provide additional rationale for investigating the role of sex hormones and autonomic dysfunction in IBS.

**Keywords:** SNP; Biobank Research; Genetics; Bowel Symptoms.

Irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorder, affecting an average of 11% people worldwide with symptoms of abdominal pain and bloating, associated with constipation, diarrhea, or their combination.<sup>1</sup> While expert consensus (Rome) criteria represent the current gold standards to characterize IBS patients based on their symptoms, the underlying pathophysiology is still poorly understood, and this hampers the implementation of adequate therapeutic strategies, their efficacy, and precision.<sup>2,3</sup> IBS is a leading cause of work absenteeism, consumes 0.5% of annual health care budgets, and the identification of biomarkers suitable for patients stratification is therefore an active area of dedicated clinical research.<sup>3</sup> In recent years, a number of factors have been proposed to contribute to IBS pathophysiology, including miscommunication along the gut-brain axis, visceral hypersensitivity and altered motility, dysregulated immune activation, bile acids malabsorption, food intolerance, and gut microbiota dysbiosis.<sup>3</sup>

Although with varying outcomes, a heritable component of IBS has long been demonstrated in independent twin and family studies<sup>4</sup> and, more recently, in a nationwide survey of first-, second-, and third-degree relatives of Swedish IBS probands.<sup>5</sup> Based on classical candidate-gene approaches,

**WHAT YOU NEED TO KNOW**

**BACKGROUND AND CONTEXT**

Genetic predisposition to irritable bowel syndrome (IBS) is recognized, though large-scale genetic studies have been lacking.

**NEW FINDINGS**

In a population-based GWAS of more than 300,000 people from UK, the researchers report female-specific association with chromosome 9q31.2, in a locus previously linked also to age at menarche.

**LIMITATIONS**

IBS cases were identified using self-reported doctor's diagnoses from questionnaire data.

**IMPACT**

This is the largest IBS genetic study, which provides additional rationale to investigate the role of sex hormones in IBS.

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previous work from our group and others has highlighted the presence of rare pathogenic *SI* (sucrose-isomaltase) and *SCN5A* (voltage-gated sodium channel NaV1.5) variants in 2%–4% of IBS patients, possibly linking their symptomatic manifestations to disaccharide intolerance and channelopathies, respectively.<sup>6,7</sup> Additional associations with common single nucleotide polymorphisms (SNP) have been reported in the past (*TNFSF15*, *TRPM8*, *CDC42*, *NPSRL*, *KDELR2*, and other genes).<sup>8–13</sup> Given the documented familial predisposition and the evidence of diverse genetic

**Abbreviations used in this paper:** AAM, age at menarche; FUMA, functional mapping and annotation; GO, gene ontology; GWAS, genome-wide association study; IBS, irritable bowel syndrome; QC, quality control; SNP, single nucleotide polymorphism.

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associations with IBS, there is hope that genetic studies will identify actionable pathways that may allow enhanced therapy for IBS.

To date, IBS genetic studies have been largely underpowered to detect modest genetic risk effects with genome-wide significance and no true unequivocal IBS risk locus has been thus far identified. Overall, large-scale gene-mapping efforts have been lacking, mainly due to the scarcity of adequately sized patient cohorts, and the heterogeneity of results observed across epidemiological studies. To try to overcome this issue, we recently proposed the use of large population-based cohorts for the study of IBS predisposition, leveraging large sample sizes and a harmonized (though less clinically informative) questionnaire-based definition of cases and controls.<sup>14</sup>

Here we report a genome-wide association study (GWAS) of IBS symptoms in the UK Biobank cohort, which includes health information and SNP genotype data from 500,000 people aged 40–69 years, recruited across the United Kingdom between 2006 and 2010. We used data on medical conditions recorded as doctor's diagnoses, self-reported by UK Biobank participants via touchscreen questionnaires and later validated by a trained nurse during follow-up interviews. Genome-wide significant findings for a locus on chromosome 9q31.2 were further investigated in multi-national case-control cohorts from tertiary centers with expertise in IBS, and from a small general population sample from Sweden. The results point to female-specific genetic risk effects on IBS associated with constipation and harder stools.

## Materials and Methods

### Study Subjects

**UK Biobank.** UK Biobank ([www.ukbiobank.ac.uk](http://www.ukbiobank.ac.uk)) is a population-based cohort study consisting of ~500,000 genotyped individuals (aged 40–69 years) recruited between 2006 and 2010 in the United Kingdom.<sup>15</sup> Each participant underwent cognitive and physical assessment and, together with genotypes, a variety of phenotypic data have been collected, including demographics and health-related information. Health data (initially collected via a touchscreen questionnaire) included previous doctor's diagnoses of non-cancer medical conditions, as self-reported by UK Biobank participants (recorded at UK Biobank as data on “non-cancer illness code, self-reported”). These were further investigated in a verbal interview by a trained nurse, who ratified and recorded the specific condition as self-reported doctor's diagnosis. For the purpose of this study, individuals with a doctor's diagnosis of IBS were selected as cases, while all other participants were regarded as controls. Before genotyping quality control (QC) and imputation, further selections criteria included (self-reported) British ancestry, and absence of independent diagnoses of inflammatory bowel disease and celiac disease (for both cases and controls) or IBS (for controls) in the data fields “non-cancer illness code, self-reported” and “diagnoses-main ICD10” from hospital inpatient stay medical records. This resulted in 9576 IBS cases and 336,499 controls included in downstream analyses (Table 1). UK Biobank received ethical approval from the

**Table 1.** Demographics of Cohorts Included in the Study

Data set	n	Age, y, mean (SD)	Female to male ratio
UK Biobank			
IBS	9576	56.4 (7.9)	7,130:2,446
Controls	336,499	56.9 (8.0)	178,076:158,423
Case-control			
IBS	2045	42.0 (14.8)	1592:453
IBS-C	598	42.0 (14.1)	536:62
IBS-D	876	42.3 (14.7)	612:264
IBS-M	502	41.8 (15.8)	401:101
Controls	7955	56.5 (16.4)	4334:3621
PopCol			
All	249	53.6 (11.4)	158:91

IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; IBS-M, IBS with constipation and diarrhea.

competent Research Ethics Committee (REC reference for UK Biobank is 11/NW/0382).

**Irritable Bowel Syndrome Cases and Controls.** *Cases.* We studied 2045 unrelated IBS patients (598 IBS with constipation, 876 IBS with diarrhea, and 502 IBS with constipation and diarrhea, according to Rome Criteria) of European ancestry from centers in Sweden (multicenter study), The Netherlands (Maastricht University), Belgium (GlaxoSmithKline multinational cohort from Translational Research in Gastrointestinal Disorders Leuven), Italy (multicenter study), and United States (Mayo Clinic and University of California-Los Angeles) (Table 1), as part of a larger ongoing collaborative study on the genetics of IBS ([www.mdalab.org/bellygenes](http://www.mdalab.org/bellygenes)). These cohorts have been extensively characterized and described in detail in previous studies.<sup>6,8–10,12,13</sup> Population outliers were removed from the analysis based on principal component analysis of available genotype data. Approval of genetic studies was obtained at respective centers from competent local ethics authorities. Karolinska Institutet's Research Ethics Committee approved the global multicenter study and the *bellygenes initiative* (protocol nr 2016/1620-31/2).

*Controls.* A total of 7955 ancestry-matched, healthy controls were randomly selected from previously published studies or general population cohorts with available genetic data. Their characteristics have been described previously and the demographics are reported in Table 1. Swedish controls were from the TwinGene subset of genotyped individuals from the Screening Across the Lifespan Twin (SALT) study.<sup>16</sup> Italian blood donor controls were described previously in a study from the International IBD Genetics Consortium.<sup>17</sup> Healthy controls from the Stroke Genetics Network study were included as Belgian controls.<sup>18</sup> UK controls were from the population-based cohort Understanding Society, a longitudinal study that follows 40,000 UK households.<sup>19</sup> The Health and Retirement Study, which includes more than 12,000 Americans aged older than 50 years, was identified as a control group for the United States.<sup>20</sup> Dutch study controls included healthy individuals from the Maastricht area,<sup>21</sup> and participants in the 500 Functional Genomics Project.<sup>22</sup> Informed consent was obtained from all IBS patients and controls at respective centers, and the global



study protocol was approved by Karolinska Institutet's Research Ethics Committee.

**Population-Based Colonoscopy Study.** The Population-Based Colonoscopy (PopCol) study is a general population-based cohort with randomly selected participants from Stockholm, Sweden, also previously described in detail and included in genetic studies.<sup>7,9,23</sup> The present study includes 249 subjects (63% females, mean age of 56.5 years) (Table 1) with available genotypes and recordings of defecation patterns (including stool consistency based on the Bristol Stool Form Scale, daily average range, 1.5–6.9), and excluding individuals with inflammatory bowel disease and celiac disease. Informed consent was obtained from all participants and the PopCol study protocol was approved by Karolinska Institutet's Research Ethics Committee (protocol no. 394/01).

### Genotyping Quality Control and Imputation

**UK Biobank.** UK Biobank full genetic data release (July 2017) was used in this study. This included genotype data for 488,377 UKB participants from 2 genotyping arrays (438,427 UK Biobank Axiom array and 49,950 UK BiLEVE Axiom array) sharing >95% content. Details of the array design, genotyping, and imputation procedures have been published elsewhere.<sup>15</sup> Both the Haplotype Reference Consortium and the UK10K + 1000 Genomes Phase 3 reference panels were used for imputation at UK Biobank, although only Haplotype Reference Consortium-imputed markers were used in this study following UK Biobank advice, because of inconsistencies in SNP marker annotation discovered for other panels. Before association testing, rigorous per-sample and per-marker QC procedures were applied, as follows: we excluded individuals with call rate <95%, genotype-phenotype sex discrepancies, deviant heterozygosity (UK Biobank outlier flag), related individuals (KING kinship coefficient > .0663) and population outliers identified via principal component analysis (absolute deviation from the median exceeding 6× interquartile range); we removed SNP markers with call rate <95% (or significant [ $P < .05$ ] call rate difference between cases and controls), poor imputation accuracy (IMPUTE2 INFO score <0.8), minor allele frequency <1%, and markers out of Hardy Weinberg equilibrium ( $P < 1.0 \times 10^{-5}$ ). This yielded high-quality data for 346,075 individuals and 7,287,191 SNP markers, showing no population stratification (scaled genomic inflation factor  $\lambda_{1000} = 1.00$ ). For the genome-wide significant locus at 9q31.2, individual cluster plots for all genotyped markers were visually inspected using Evoker 2.4.1 ([www.sanger.ac.uk/science/tools/evoker](http://www.sanger.ac.uk/science/tools/evoker)) to ensure correct allele calling and absence of genotyping artifacts or batch effects.

**Case-Control and PopCol rs10512344 Genotype Data.** For IBS cases and controls, rs10512344 genotypes were extracted from currently available imputed genotype data. IBS patients were genotyped on Illumina Infinium CoreExome24 (Illumina, San Diego, CA) as part of the *bellygenes initiative* project ([www.mdalab.org/bellygenes](http://www.mdalab.org/bellygenes)), while controls genotypes were obtained from previous Illumina genotyping efforts at respective individual sites and data sources (study principal investigators or biobanks). The rs10512344 data were extracted from imputed genotypes, obtained after QC and imputation (including harmonization of genotypes from different

platforms using GenotypeHarmonizer; [github.com/molgenis/systemsgenetics/wiki/Genotype-Harmonizer](https://github.com/molgenis/systemsgenetics/wiki/Genotype-Harmonizer)), following a pipeline similar to that applied to UK Biobank data. The rs10512344 genotype data included in this study were all imputed with high accuracy (IMPUTE2 INFO score range, 0.977–0.992). PopCol rs10512344 genotypes were extracted from available QC and imputed Illumina OmniExpressExome-8 v1 genotype data, which have already been used and described in previous publications.<sup>7,9</sup> Imputation metrics ensured high imputation accuracy at this marker (IMPUTE2 INFO score = 1.0).

### Genome-Wide Association Studies and Follow-Up Analyses

**UK Biobank Genome-Wide Association Studies.** Data from 346,075 UK Biobank individuals (9576 IBS cases and 336,499 controls) entered the regression model. Association tests were performed in PLINK2 ([www.cog-genomics.org/plink/2.0/](http://www.cog-genomics.org/plink/2.0/)) using logistic regression under an additive genetic model, adjusting for sex, age, genotyping array, and the first 10 principal components from principal component analysis. Markers showing suggestive association ( $P < 5 \times 10^{-6}$ ) were further analyzed through functional genomics annotation in order to map and prioritize genes (see next section). UK Biobank GWAS results were reported on the Manhattan plot using the Bioconductor *GWASTools* package ([bioconductor.org/packages/release/bioc/html/GWASTools.html](http://bioconductor.org/packages/release/bioc/html/GWASTools.html)).

**Post Genome-Wide Association Study Functional Genomics Analyses.** IBS risk loci were defined as non-overlapping genomic regions extending a linkage disequilibrium window ( $r^2 = 0.6$ ) from the association signals with  $P < 5.0 \times 10^{-6}$ . Annotation of GWAS results, including genes mapping to the identified risk loci, was performed with functional mapping and annotation (FUMA) of GWAS ([fuma.ctglab.nl/](http://fuma.ctglab.nl/)).<sup>24</sup> Integrating data from multiple biorepositories, genes are prioritized by FUMA via positional mapping based on annotations obtained from ANNOVAR ([annovar.openbioinformatics.org](http://annovar.openbioinformatics.org)), potential regulatory functions (15-core chromatin state predicted by ChromHMM ([compbio.mit.edu/ChromHMM](http://compbio.mit.edu/ChromHMM)) for 127 tissue/cell types), and effects on gene expression using expression quantitative trait loci of several tissue types from BIOS QTL ([molgenis58.target.rug.nl/biosqtlbrowser](http://molgenis58.target.rug.nl/biosqtlbrowser)), BRAINEAC ([www.braineac.org](http://www.braineac.org)) and GTEx ([www.gtexportal.org](http://www.gtexportal.org)) databases. To test for over-representation of biological functions based on gene annotations (gene set enrichment analysis), we screened the Molecular Signature Database (MsigDB, including hallmark gene sets, curated gene sets, motif gene sets, Gene Ontology [GO] gene sets, oncogenic signatures, and immunologic signatures, [software.broadinstitute.org/gsea/msigdb](http://software.broadinstitute.org/gsea/msigdb)) using the list of FUMA mapped genes against a total of 19,264 protein-coding genes in hypergeometric enrichment tests. Gene sets with an adjusted  $P < .05$  (false discovery rate correction according to Benjamini-Hochberg) were considered significant evidence of enrichment.

**9q31.2 Locus-Specific Analyses.** UK Biobank sex-stratified analyses at the genome-wide significant locus 9q31.2 were performed as in the whole GWAS (excluding sex as covariate). Global and sex-stratified regional plots of 9q31.2 GWAS association signals were produced

with *ggplot2* ([cran.r-project.org/web/packages/ggplot2/](http://cran.r-project.org/web/packages/ggplot2/)). Haplotype associations testing (SNP markers rs10512344 and rs10156597) for IBS in UK Biobank was performed using logistic regression and age, array and top 10 principal components as covariates with *haplo.stats* ([cran.r-project.org/web/packages/haplo.stats/](http://cran.r-project.org/web/packages/haplo.stats/)). For the purpose of conducting colocalization and conditional analyses, we extracted 9q31.2 locus information from publicly available summary statistics data reported in the latest published age at menarche (AAM) GWAS meta-analysis,<sup>25</sup> performed by the ReproGen consortium ([www.reprogen.org](http://www.reprogen.org)). In order to investigate the interdependence of AAM and IBS association signals, 2 summary statistics-based approaches were used: multi-trait-based conditional analysis using the Mendelian Randomization approach implemented in mtCOJO from the genome-wide complex trait analysis tools,<sup>26</sup> and Approximate Bayes Factor colocalization analysis to test whether the same or different causal variants are associated with multiple traits (IBS/AAM) using *coloc* ([cran.r-project.org/web/packages/coloc/](http://cran.r-project.org/web/packages/coloc/)). The SNP marker rs10512344 was tested for association with IBS and stool consistency in additional cohorts (case–controls cohorts from tertiary centers and PopCol) in sex-stratified analyses: one-tailed association test with IBS in cases and controls was carried out on the combined cohorts using PLINK with logistic regression under an additive genetic model adjusted for age and country of origin; correlation between genotype and average Bristol Stool Form Scale scores was tested using linear regression methods implemented in R ([cran.r-project.org](http://cran.r-project.org)) adjusting for age.

## Results

### Genome-Wide Association Study of Irritable Bowel Syndrome in UK Biobank

For GWAS purposes, we exploited the full release (July 2017) of UK Biobank data, including health and genotype information from 488,377 participants. IBS cases were identified based on doctor's diagnoses self-reported by UK Biobank participants (collected via touchscreen questionnaire and ratified during a follow-up interview with a trained nurse; see Materials and Methods), while the remainder of the cohort was assigned to the control group. After exclusion of other relevant gastrointestinal diseases, people of non-British ancestry, related individuals, and individuals with missing data, we identified 9576 IBS cases and 336,499 controls (Table 1). We applied stringent per-sample and per-marker QCs to SNP data from UK Biobank (including both observed and imputed genotypes) in order to remove low-quality DNA and rare or poor-performing SNPs, and to identify and exclude population outliers based on principal components analysis of QC-filtered genotype data (Materials and Methods). This resulted in high-quality data for a total of 7,287,191 SNP markers, which were tested for association with IBS using age-, sex-, genotyping array-, and principal component-adjusted logistic regression under an additive genetic model (Materials and Methods). The GWAS analysis yielded association signals of suggestive ( $P < 5.0 \times 10^{-6}$ ) significance for 14 independent loci, including a

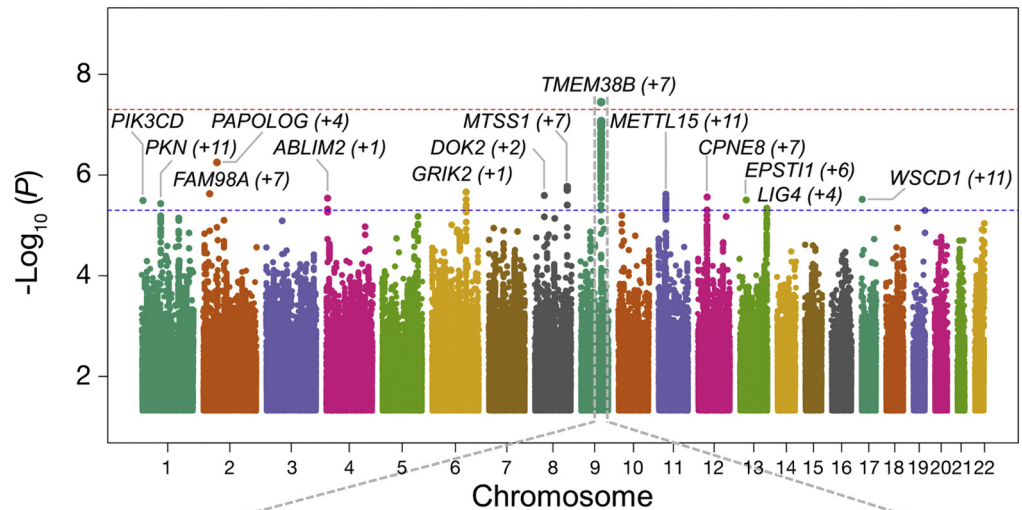
genome-wide significant ( $P < 5.0 \times 10^{-8}$ ) locus on chromosome 9q31.2 (rs10512344 [minor allele C]  $P = 3.57 \times 10^{-8}$ ,  $\beta = .190$ ) (top panel in Figure 1, and Table 2).

### Functional Annotation of Genome-Wide Association Study Results

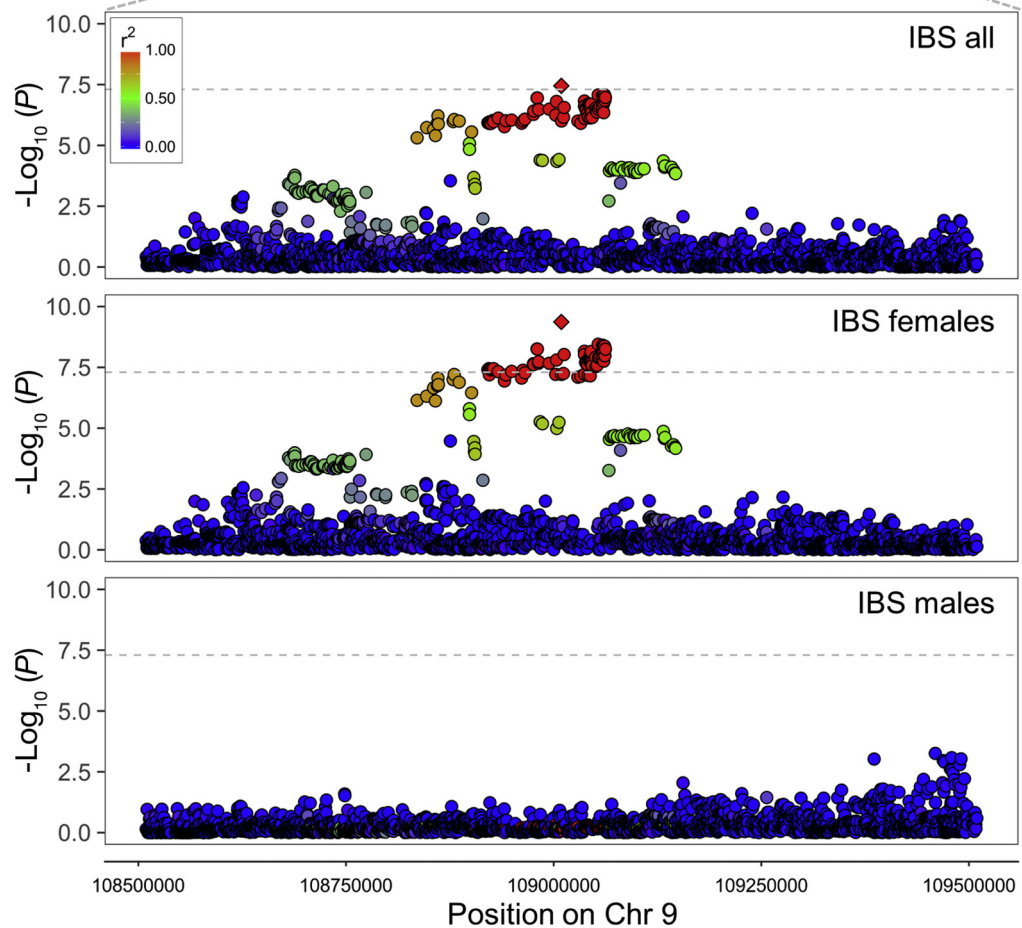
To gain biological insight from the observed associations, we used Functional Mapping and Annotation of GWAS (FUMA GWAS)<sup>24</sup> for computational annotation and interpretation of results (Materials and Methods). Based on genomic coordinates, regional linkage disequilibrium, presence of expression quantitative trait loci, and chromatin interactions, FUMA GWAS mapped a total of 93 genes to the 14 risk loci identified (Figure 2A and Table 2). These genes showed overall heterogeneity of mRNA expression across different human tissues from GTEx database (<http://www.gtexportal.org>), with a few transcripts showing higher expression in the brain (eg, *BARHL2*, *GRIK2*) and intestine (eg, *CLCA1*, *CLCA4*) (data not shown). A gene set enrichment analysis (see Materials and Methods) revealed, among other things, that the IBS risk gene pool is significantly enriched for *intracellular calcium activated chloride channel activity*, *ion gated channel activity*, and *anion channel activity* among the GO terms of the Molecular Function domain, as well as for targets of microRNA species from the miR-15 family (Figure 2B).

### 9q31.2 Locus-Specific Analyses

The genome-wide significant 9q31.2 locus (see Materials and Methods for additional QC of genotype data from this region) has also been consistently associated with AAM in several GWAS studies,<sup>25,27</sup> and it is known that IBS prevalence in women is higher than in men.<sup>1</sup> To test for potential sex-specific genetic risk effects, we repeated our association analysis upon stratification of UK Biobank participants in male (2446 cases and 158,423 controls) and female (7130 cases and 178,076 controls) groups. As shown in Figure 1 (bottom panel), this revealed the association signal to be absent in males (rs10512344;  $P = .79$ ), while it is entirely accounted for by the female group, where both the strength of association and the magnitude of risk effects increased, despite the reduction in sample size (rs10512344;  $P = 4.29 \times 10^{-10}$ ;  $\beta = .245$ ) (Table 3). The 9q31.2 SNP with strongest genetic risk effects in AAM (rs10156597;  $P = 3.70 \times 10^{-107}$  in the most recent meta-analysis of AAM GWAS<sup>25</sup>) is not in linkage disequilibrium ( $r^2 = 0.04$ ) with the top IBS risk marker rs10512344, and the analysis of their haplotypic combinations in UK Biobank data suggests the association with IBS is not dependent on the AAM-effect allele (Figure 3A). An AAM-conditioned analysis of the entire 9q31.2 region, using the latest AAM GWAS meta-analysis summary statistics with mtCOJO (Materials and Methods) did not disclose a significant reduction of the IBS signal of association based upon AAM conditioning (Figure 3B). Finally, approximate Bayes factor colocalization analysis (Materials and Methods) provided further evidence for AAM independence of the IBS association



**Figure 1.** Manhattan plot of GWAS results and regional plots for the 9q31.2 locus. *Top panel:* GWAS association signals ( $-\log_{10} P$ ) are reported for SNP markers across all chromosomes, shown in alternate colors. Significance levels corresponding to genome wide ( $P = 5.0 \times 10^{-8}$ ) and suggestive ( $P = 5.0 \times 10^{-6}$ ) thresholds are indicated, respectively, with red and blue horizontal dashed lines. For each independent association signal, the nearest gene (mapping closest to the tag SNP) is reported, together with the number of additional genes from the same locus (in brackets). *Bottom panel:* 9q31.2 locus-specific regional plots of association signals in the entire UK Biobank cohort, and in sex-stratified analyses. SNPs are color-coded to reflect their linkage disequilibrium ( $r^2$ ) with the tag SNP rs10512344 (diamond symbol) according to the color key in the upper left corner.



signal (9q31.2 locus associated with both traits but with different causal variants, posterior probability for  $H_3 = 99.98\%$ ).

### 9q31.2 Follow-Up Analyses

We further characterized the 9q31.2 association through additional analyses in independent datasets,

including Rome Criteria-defined IBS patients recruited at expert neurogastroenterology tertiary centers: we extracted genotype data for 2045 cases and 7955 population-based controls of European descent (Materials and Methods) currently available from an ongoing large-scale IBS genetics project ([www.mdalab.org/bellygenes](http://www.mdalab.org/bellygenes)), and tested the rs10512344 marker again in sex-stratified analyses. As reported in Table 3, we

**Table 2.** Genome-Wide Association Study Results Including Functional Mapping and Annotation Annotations

SNP	Chr	EA	OA	EAF cases	EAF controls	INFO	$\beta^a$	SE	P value	Positional map <sup>b</sup>	eQTL map <sup>c</sup>	Chromatin interactions map <sup>d</sup>
rs140532807	1p36.22	A	G	0.015	0.011	0.89	.289	.062	$3.22 \times 10^{-6}$	<i>PIK3CD</i>		
rs10923043	1p22.2	T	C	0.565	0.548	0.99	.069	.015	$3.70 \times 10^{-6}$		<i>KYAT3</i>	<i>COL24A1, ODF2L, CLCA2, CLCA1, CLCA4, SH3GLB1, SEP15, HS2ST1, LMO4, PKN2, BARHL2</i>
rs116449111	2p22.3	C	G	0.974	0.979	0.97	-.218	.046	$2.36 \times 10^{-6}$	<i>FAM98A</i>		<i>CAPN13, GALNT14, TTC27, LTBP1, RASGRP3, CRIM1, FEZ2, FAM98A</i>
rs116781238	2p16.1	A	G	0.056	0.048	0.95	.161	.032	$5.61 \times 10^{-7}$			<i>VRK2, PAPOLG, PUS10, PEX13, AHS2</i>
rs1105615	4p16.1	T	C	0.173	0.160	0.99	.091	.02	$2.88 \times 10^{-6}$	<i>ABLIM2</i>		<i>SORCS2</i>
rs13212302	6q16.3	T	C	0.573	0.590	0.99	-.071	.015	$2.18 \times 10^{-6}$			<i>ASCC3, GRIK2</i>
rs117107090	8p21.3	A	G	0.975	0.980	0.95	-.225	.048	$2.55 \times 10^{-6}$			<i>LZTS1, DOK2, XPO7</i>
rs4871509	8q24.13	A	G	0.463	0.446	0.99	.071	.015	$1.69 \times 10^{-6}$	<i>MTSS1</i>	<i>TRMT12, NDUFB9, MTSS1</i>	<i>TATDN1, ZNF572, SQLE, KIAA0196, NSMCE2, MTSS1, TRMT12, NDUFB9</i>
rs10512344	9q31.2	C	G	0.048	0.040	0.98	.190	.034	$3.57 \times 10^{-8}$			<i>OR13C8, TAL2, TMEM38B, ZNF462, RAD23B, ELP1, FAM206A, TMEM245</i>
rs925870	11p14.1	T	C	0.860	0.848	0.99	.100	.021	$2.41 \times 10^{-6}$			<i>ANO3, MUC15, BBOX1, CCDC34, LGR4, LIN7C, BDNF, KIF18A, METTL15, KCNA4, FSHB, ARL14EP</i>
rs61939612	12q12	T	G	0.390	0.373	0.97	.072	.015	$2.74 \times 10^{-6}$	<i>CPNE8</i>	<i>CPNE8</i>	<i>ALG10B, KIF21A, ABCD2, C12orf40, SLC2A13, MUC19, PDZRN4, CPNE8</i>
rs1540951	13q14.11	A	G	0.318	0.334	0.98	-.075	.016	$3.14 \times 10^{-6}$	<i>EPSTI1</i>	<i>DNAJC15, EPSTI1</i>	<i>AKAP11, FAM216B, ENOX1, CCDC122, LACC1, EPSTI1, DNAJC15</i>
rs147085754	13q33.3	A	C	0.030	0.025	0.99	.198	.043	$4.57 \times 10^{-6}$	<i>LIG4, FAM155A</i>		<i>DAOA, ARGLU1, ABHD13, LIG4, FAM155A</i>
rs146313367	17p13.2	A	C	0.084	0.075	0.97	.125	0.027	$3.04 \times 10^{-6}$	<i>WSCD1</i>		<i>NLRP1, AIPL1, FAM64A, PITPNM3, KIAA0753, TXNDC17, MED31, C17orf100, XAF1, FBXO39, TEKT1</i>

Chr, chromosome and cytogenetic band; EA, effect allele; EAF, effect allele frequency; eQTL, expression quantitative trait loci; INFO, imputation quality score; OA, other allele; SE, standard error.

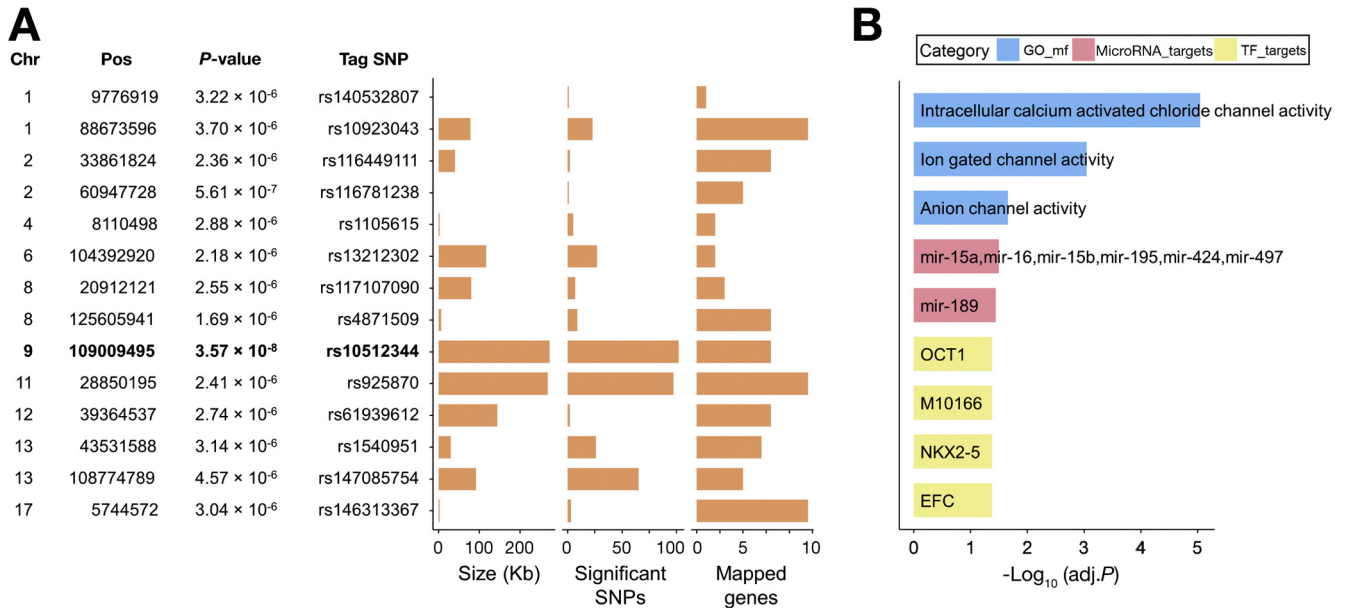
<sup>a</sup>Regression coefficient.

<sup>b</sup>Genes physically mapped within the region.

<sup>c</sup>Genes mapped through eQTL effects.

<sup>d</sup>Genes mapped through chromatin interactions.





**Figure 2.** FUMA analysis of GWAS results. (A) GWAS risk loci ( $P < 5.0 \times 10^{-6}$ ) with tag SNPs and FUMA annotations. The genome-wide significant locus 9q31.2 is highlighted in bold. (B) Gene set enrichment analysis significant findings (ranked based on false discovery rate-adjusted  $P$  value) and color-coded categories GO molecular function (GO\_mf), miRNA targets (microRNA\_targets), and transcription factor targets from the Molecular Signature Database (TF\_targets).

observed genetic risk effects in the same direction and of the same magnitude as in the GWAS and, despite the relatively small sample size (>4000 IBS cases would be needed to replicate 9q31.2 GWAS findings with adequate statistical power >80%), we detected a significant female-specific association in the constipation-predominant IBS group ( $P = .015$ ;  $\beta = .383$ ). Finally, analysis of genotype and bowel diary data available for 249 individuals from the PopCol population-based cohort (Materials and Methods) revealed that the IBS/constipation risk allele rs10512344 C also strongly correlates with harder stools in females ( $P = .0012$ ,  $\beta = -1.105$ ) (Table 3).

### Discussion

We report here the first powered GWAS of IBS, implemented by taking advantage of available health and genotype data from the large UK Biobank resource, which includes approximately half a million participants. Through the analysis of 7,287,191 SNP markers in 9,576 cases and 336,499 controls, we identify 1 genome-wide significant risk locus on chromosome 9q31.2, as well as 13 additional loci of suggestive significance that represent ideal candidates for future targeted studies in other large independent cohorts. The large sample size, the nested case-control design, and the rigorous per individual/per genotype data

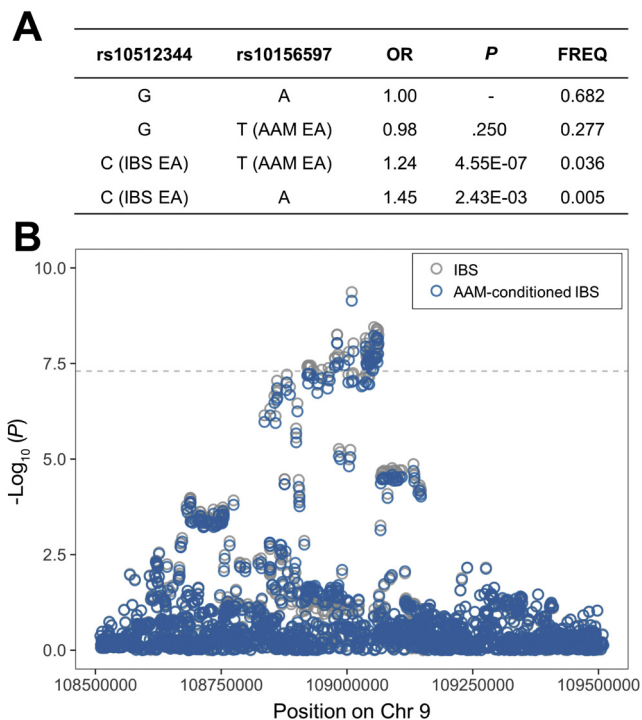
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**Table 3.** Sex-Stratified Association Results for the Single Nucleotide Polymorphism rs10512344

Data set tested trait	EAF	Females			Males		
		$\beta$	SE	$P$ value	$\beta$	SE	$P$ value
UK Biobank							
IBS	0.0404	<b>.245</b>	<b>.039</b>	<b>4.29 × 10<sup>-10</sup></b>	.019	.073	.789
Case-control							
IBS	0.0362	<b>.228</b>	<b>.127</b>	<b>.036</b>	.192	.183	.147
IBS-C	0.0357	<b>.383</b>	<b>.177</b>	<b>.015</b>	.256	.428	.274
IBS-D	0.0348	.008	.190	.483	.166	.224	.229
IBS-M	0.0342	.232	.204	.255	-.120	.388	.757
PopCol							
BSFS score, mean	0.0241	<b>-1.105</b>	<b>.335</b>	<b>.0012</b>	.291	.387	.455

NOTE. Bold type indicates significant associations ( $P$  value < .05). BSFS, Bristol Stool Form Scale; EAF, effect allele frequency; IBS-C, IBS with constipation; IBS-D, IBS with diarrhea; IBS-M, IBS with constipation and diarrhea; SE, standard error.





**Figure 3.** Haplotype association and conditional analysis. (A) Summary statistics from association tests between IBS/AAM tag SNPs haplotypes and IBS in UK Biobank (females only). FREQ, haplotype frequency; OR, odds ratio; P, P value. (B) Regional plot of the 9q31.2 locus before and after conditional analysis on AAM. The horizontal dashed line shows the genome-wide significance P value threshold ( $5.0 \times 10^{-8}$ ). Gray and blue circles correspond to unconditioned and AAM-conditioned P values, respectively.

QC pipeline are among the major strengths of this study, while a limitation results from the selection of cases and controls based on indirect evidence of a clinical diagnosis (patients report that a doctor has diagnosed them with IBS via touchscreen questionnaire and during a follow-up interview with a trained nurse). However, because of the remarkable opportunity deriving from increased sample size (questionnaire data are available for several large population-based cohorts), the use of similar self-reported traits and conditions has been increasingly and successfully applied in many recent GWAS, including GWAS of common diseases in UK Biobank ([www.ukbiobank.ac.uk/genetic-publications](http://www.ukbiobank.ac.uk/genetic-publications)). In addition, evidence of replication is provided for the 9q31.2 locus in a multi-national cohort of 2045 Rome Criteria-based IBS cases from tertiary centers with expertise in IBS; this corroborates initial GWAS findings and highlights the need to assemble large cohorts suitable for clinical validation of genetic findings.

GWAS-downstream analyses and functional annotation of risk loci with FUMA show that the IBS risk pool is significantly enriched for targets of microRNAs from the miR-15 family, as well as for genes involved in cellular activities mediated by ion channels. Members of the miR-15 family have been reported to affect the expression of serotonin receptors involved in the regulation of motor function (miR-16) and epithelial barrier integrity (miR-16

and miR-125b), and their dysregulated expression has been recently associated with clinical symptoms in IBS patients.<sup>28</sup> The link to ion channel biology adds to emerging evidence for a role of channelopathies in IBS pathophysiology.<sup>29</sup> Previous studies have identified IBS risk effects due to rare and common variants in the ion channel genes *SCN5A* (the voltage-gated sodium channel  $Na_v1.5$  expressed on the myenteric plexus interstitial cells of Cajal)<sup>6,30</sup> and *TRPM8* (the “cold” receptor target of menthol and other intestinal smooth muscle relaxants),<sup>9</sup> and have also highlighted ion channel activity among the GO biological pathways most relevant to the control of stool frequency.<sup>31</sup> In particular, the specific GO term with strongest evidence of enrichment in the current study (*intracellular calcium activated chloride channel activity*, false discovery rate-adjusted  $P = 9.10 \times 10^{-6}$ ) includes 4 calcium-dependent chloride channels at 2 risk loci on chromosome 1p22.2 (*CLCA1*, *CLCA2*, and *CLCA4*) and 11p14.1 (*ANO3*). Among other functions, these channels play important roles in intestinal fluid secretion and secretory diarrhea (*CLC1/2/4*), as well as in the control of gut peristalsis mediated by the interstitial cells of Cajal (*ANO3*). These are interesting observations that may provide rationale for future translational opportunities, because ion channels are attractive drug targets accessible on the cell surface and represent potential gateways to therapeutically actionable pathways. Nevertheless, the computational annotation of IBS GWAS results needs to be interpreted with caution because it mostly includes regions of suggestive significance (13/14 loci) that may reveal false positives in replication analyses and it may be driven by fewer enriched loci with many genes from the same GO term category (while possibly only 1 gene/locus ultimately mediates specific risk effects). At least the latter does not appear to vitiate our FUMA results for ion channel enrichment (still highly significant under a conservative assumption of only 1 causative gene per locus, not shown).

The identification of a genome-wide significant risk locus for IBS is the main finding from our study. The association at 9q31.2 is tagged by the SNP marker rs10512344, whose risk allele C is not common in UK Biobank and other populations of European ancestry (4% in UK Biobank, and 2%–5.1% in other EU reference cohorts, [www.ensembl.org](http://www.ensembl.org)), therefore, this variant will only affect IBS risk in a small proportion of individuals. However, while genetic risk scores (including rs10512344 C) may be exploited later for stratifying patients into different treatment groups when more GWAS data are accumulated, the essential value of these early findings is in their interpretation for pathway discovery. The observation of female-specific IBS risk effects, together with its known association with AAM, make the 9q31.2 locus particularly interesting. Although not previously detected in independent cohort studies of similarly large sample size,<sup>32</sup> Day et al<sup>33</sup> recently reported an association between IBS and early AAM in UK Biobank, which raises the question as to whether our genetic findings may reflect this overlap (ie, AAM genetic risk effects also drive, at least in part, the association with IBS). We have shown this is not the case by applying 3 alternative methodological approaches,

demonstrating that disease association signals do not colocalize at the 9q31.2 locus, where IBS risk variants differ from AAM risk variants. It is noteworthy that the association between early AAM and IBS is of borderline significance in UK Biobank, a similar trend was also observed for late AAM, and significant early/late AAM associations were detected for other 48 adverse outcomes across a range of cancer, cardiometabolic, gynecological/obstetric, gastrointestinal, musculoskeletal, and neurocognitive categories.<sup>33</sup> Therefore, although the association with young AAM and IBS may not be unequivocal, it does not appear to be specific.

Nevertheless, irrespective of specific associations with individual risk variants, the 9q31.2 region has been linked to a series of human conditions and traits (voice breaking in males, body mass index, waist circumference, breast and prostate cancer; GWAS catalog [www.ebi.ac.uk/gwas/](http://www.ebi.ac.uk/gwas/)) that, like AAM, are under the influence of hormonal stimuli, especially those involving mechanisms of action of sex hormones. This is particularly interesting in the context of IBS because of the known sex differences in its prevalence, symptomatic manifestations, response to treatment and, possibly, pathophysiology. While IBS symptoms are twice as common in women at the level of the general population, the female to male ratio increases to 5:1 in patients from specialized tertiary centers,<sup>34</sup> where better treatment outcomes have also been observed, for instance, in the colonic transit response of women treated with alosetron (5-HT<sub>3</sub> receptor antagonist) compared to men.<sup>35</sup> In addition, colonic transit appears to be generally slower in women compared to men,<sup>36</sup> and female IBS patients most often report symptoms of constipation and a feeling of incomplete evacuation (opposite to men who more frequently suffer from diarrhea).<sup>37</sup> Our targeted 9q31.2 follow-up analyses are in line with these observations, as the rs10512344 risk genotype primarily associated with constipation-predominant IBS in female cases from tertiary centers, and correlated with harder stools in women but not men from a population-based cohort.

Increasing female preponderance of IBS is detected from the time of puberty to adult age, therefore, a pathogenetic role of sex hormones has been postulated and actively investigated in IBS. In addition, several chronic pain disorders often overlapping with IBS (chronic fatigue syndrome, fibromyalgia, migraine, chronic pelvic pain, and others) show increased prevalence in women and correlation of symptoms with hormonal status.<sup>38,39</sup> Sex hormones contribute important peripheral and central regulatory signals along the gut-brain axis, directly and indirectly (via other hormones) affecting gut motor and sensory functions, permeability, and mucosal immune activation.<sup>39,40</sup> In particular, estrogens and their receptors (which contribute to induce menarche) show wide and abundant expression in the central and enteric nervous systems, and interact with other hormones, such as serotonin and corticotropin-releasing factor, which are known to mediate visceral responses to stress among other functions.<sup>41</sup> The effect of sex hormones on gastrointestinal function is probably best typified by the fluctuations of bowel behavior with the different phases of the menstrual cycle,<sup>39,42</sup> and the

appearance of symptoms perimenstrually in otherwise healthy women.<sup>43</sup> Ovarian hormone variations have been linked not only to changes in sensorimotor gastrointestinal function, but also to hyper-responsiveness to stress with potential repercussions on gut immune activation and barrier function, and the emotional system responsible for visceral pain perception.<sup>43,44</sup> Of note, female IBS patients have been shown to be affected by menstruation to a greater degree than healthy women,<sup>39,45</sup> with exacerbation of bowel symptoms possibly due to their reported reduced rectal sensitivity at the time of menses.<sup>46</sup>

While the 9q31.2 association signal overlaps with an intergenic region, FUMA GWAS analysis mapped 8 genes to this risk locus based on functional annotation of chromatin interactions (Table 2). Most of these genes are either poorly characterized (*ORF13C8*, *ZNF462*, *FAM206A*, *TMEM245*), or they have a potential role in cancer (*TAL2*, *RAD23B*) and osteogenesis imperfecta (*TMEM38B*). Hence, the gene *ELP1* (elongator complex protein 1 gene, also known as *IKBKAP*) may represent the current best candidate to impact IBS risk at 9q31.2. *ELP1* codes for a protein that is part of the RNA polymerase II elongator complex involved in the control of RNA transcription via chromatin remodeling and histone acetylation.<sup>47</sup> It is expressed primarily in the adrenal and pituitary glands, the ovary, and the cerebellum ([www.gtexportal.org/home/gene/IKBKAP](http://www.gtexportal.org/home/gene/IKBKAP)), which may suggest hormonal regulation (including distal effects on chromatin rearrangements as described for estrogen receptors and other sex hormones).<sup>48</sup> Most notably, *ELP1* (*IKBKAP*) mutations cause familial dysautonomia, a recessively inherited disease due to impaired development of sensory, sympathetic, and parasympathetic neurons.<sup>49</sup> Familial dysautonomia patients typically experience sensory neuropathy characterized by pain insensitivity, gastrointestinal dysmotility, altered temperature sensation, and cardiovascular instability. Of interest, in view of the findings reported here, female familial dysautonomia patients often also show delayed AAM and a very high prevalence of premenstrual symptoms.<sup>50</sup> Autonomic response to visceral stressors is dysregulated in IBS patients,<sup>51</sup> and sex differences in autonomic nervous system reactivity to colorectal distention have been reported.<sup>52</sup> At the same time, the increased prevalence of comorbidities also characterized by autonomic dysfunction (such as the joint hypermobility syndrome) is increasingly recognized in IBS patients. Therefore, should *ELP1* be the causative gene at the 9q31.2 locus, it is possible to speculate that genotype-driven alterations of its function or expression may also contribute to IBS pathogenesis, possibly involving biological mechanisms under the control of sex hormones. Overall, the findings reported here open up for new lines of research in IBS and constipation, and warrant further investigation at the clinical and experimental levels.

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#### Conflicts of interest

The authors disclose no conflicts.

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