TRPM8 polymorphisms associated with increased risk of IBS-C and IBS-M

Recently in *Gut*, genetic variation affecting ion channels activity has been highlighted in relation to bowel function and the biology of stool frequency.[1] It is also known that 2% of patients with IBS carry functional missense mutations in the voltage-gated channel $Na_V1.5$ (*SCN5A* gene).[2] Hence, channelopathies represent potential abnormalities underlying GI dysfunction and IBS. We inspected data from our previous genome-wide association study (GWAS) of IBS,[3] in relation to 27 genes whose ion channel products contribute to GI sensorimotor development and function, visceral sensation and GI motility (see online supplementary table S1). Significant (uncorrected) results were detected for four genes (calcium voltage-gated channels *CACNA1A* and *CACNA1E*, and transient receptor potential channels *TRPV3* and *TRPM8*; see online supplementary figure S1), which were selected for replication analyses in an independent set of IBS cases (N=386) and controls (N=357) (see online supplementary material methods). A sex-adjusted logistic regression analysis of genotype data from this cohort (see online supplementary material methods) detected significant associations for *TRPM8*, and a meta-analysis of GWAS and replication yielded (i) strongest evidence of association at this locus, (ii) no statistical heterogeneity (Cochran's Q test $p>0.05$) and (iii) same direction of genetic risk effects in the two studies (table 1). Rome III IBS-subtype information available for the replication cohort revealed *TRPM8* single nucleotide polymorphisms (SNP) to impact IBS risk exclusively in the IBS-C and IBS-M types, with strongest evidence obtained from their

Table 1 Associations between IBS, IBS subtypes and *TRPM8* SNPs

		IBS (GWAS+this study)*						IBS subtypes (this study)*							
		GWAS (534/4932)		Replication (386/357)		Meta-analysis (920/5289)		IBS-D (127/357)		IBS-C (95/357)		IBS-M (163/357)		IBS-C+M (258/357)	
SNP	MA(F)†	p Value	OR	p Value	OR	p Value	OR	p Value	OR	p Value	OR	p Value	OR	p Value	OR
rs10519356	T(0.083)	**5.1E-03**	1.35	1.3E-01	1.36	**1.5E-03**	1.35	9.0E-01	0.96	**4.4E-02**	1.79	8.5E-02	1.52	**3.4E-02**	1.59
rs10166942	C(0.196)	**9.5E-03**	1.23	**9.2E-04**	1.62	**1.3E-04**	1.30	3.8E-01	1.19	**6.7E-03**	1.83	**2.8E-04**	1.90	**5.1E-05**	1.91
rs2362290	A(0.196)	**9.0E-03**	1.23	**7.3E-04**	1.64	**1.1E-04**	1.31	3.5E-01	1.21	**5.6E-03**	1.86	**2.3E-04**	1.92	**3.9E-05**	1.94
rs1003757	A(0.087)	**4.3E-03**	1.35	1.2E-01	1.37	**1.1E-03**	1.35	8.3E-01	0.94	5.3E-02	1.73	6.9E-02	1.54	**2.7E-02**	1.61
rs1003756	T(0.087)	**4.3E-03**	1.35	1.2E-01	1.37	**1.2E-03**	1.35	8.3E-01	0.94	5.3E-02	1.73	6.9E-02	1.54	**2.7E-02**	1.61
rs4497869	A(0.107)	**1.2E-02**	1.28	**3.2E-02**	1.48	**1.3E-03**	1.32	6.2E-01	1.14	**3.9E-02**	1.73	**3.1E-02**	1.60	**1.1E-02**	1.66
rs6431648	A(0.208)	**5.0E-03**	1.24	**4.0E-04**	1.65	**3.9E-05**	1.32	2.3E-01	1.26	**8.9E-03**	1.75	**2.4E-04**	1.88	**3.9E-05**	1.90
rs758277	T(0.430)	**6.1E-03**	0.83	6.7E-01	0.95	**9.6E-03**	0.86	2.8E-01	1.18	2.7E-01	0.82	3.9E-01	0.88	1.9E-01	0.84
rs758276	G(0.207)	**5.6E-03**	1.24	**4.9E-04**	1.64	**4.9E-05**	1.32	2.2E-01	1.27	**8.7E-03**	1.75	**4.3E-04**	1.83	**6.0E-05**	1.87
rs758275	G(0.206)	**5.5E-03**	1.24	**5.4E-04**	1.63	**5.0E-05**	1.32	2.2E-01	1.27	**1.1E-02**	1.73	**4.3E-04**	1.83	**7.0E-05**	1.86
rs6711120	A(0.208)	**6.0E-03**	1.24	**4.9E-04**	1.64	**5.2E-05**	1.32	2.2E-01	1.27	**8.7E-03**	1.75	**4.3E-04**	1.83	**6.0E-05**	1.87
rs9646720	G(0.204)	**5.0E-03**	1.25	**1.0E-03**	1.60	**5.9E-05**	1.32	2.2E-01	1.28	**2.7E-02**	1.62	**5.9E-04**	1.82	**1.9E-04**	1.80
rs7595960	A(0.110)	**6.5E-03**	1.29	**3.5E-02**	1.48	**7.5E-04**	1.33	6.0E-01	1.14	**4.7E-02**	1.70	**3.5E-02**	1.60	**1.3E-02**	1.64
rs7577157	A(0.111)	**7.3E-03**	1.29	**3.5E-02**	1.48	**8.4E-04**	1.33	6.0E-01	1.14	**4.7E-02**	1.70	**3.5E-02**	1.60	**1.3E-02**	1.64

*For each analysis, N size is reported in brackets (cases/controls).
†MA(F)=minor tested allele and its frequency (F); significant p values in bold type.
GWAS, genome-wide association study.

Correlation between *TRPM8* genotype and BSFS scores

SNP	Test (minor) allele	Genetic risk effect in IBS	Allele frequency	P	Rho
rs10519356	T	predisposing	0,083	**2,9E-02**	-0,174
rs10166942	C	predisposing	0,208	**3,3E-02**	-0,169
rs1003757	A	predisposing	0,095	**3,5E-02**	-0,166
rs1003756	T	predisposing	0,095	**3,9E-02**	-0,162
rs6431648	A	predisposing	0,212	**7,7E-03**	-0,221
rs758277	T	protecting	0,417	**1,5E-02**	0,199
rs7577157	A	predisposing	0,107	**2,3E-02**	-0,184

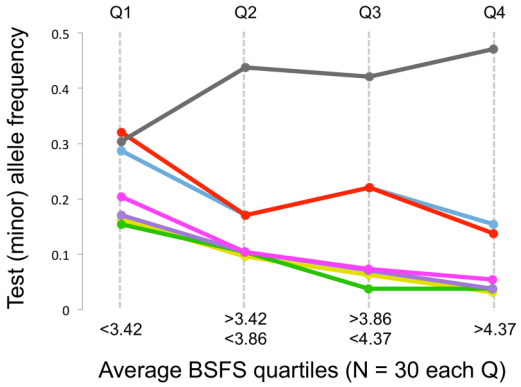

Figure 1 Correlation between *TRPM8* genotype and average Bristol Stool Form Scale (BSFS) scores. Left: Spearman correlation statistics. Right: frequency of *TRPM8* alleles across BSFS quartile groups (alleles from each SNP are colour-coded as in the table on the left).

combined analysis (table 1). This prompted us to investigate the relationship between *TRPM8* and GI motility in the Population-based Colonoscopy study (PopCol),[4] focusing on IBS-free individuals (N=120) who kept weekly records of their defaecation episodes based on the Bristol Stool Form Scale (BSFS; see online supplementary material methods). A Spearman correlation analysis revealed all IBS-C/M risk alleles to be consistently associated with harder stools, showing progressively decreasing frequencies from lower to upper average BSFS quartile groups, while opposite results were obtained for protective alleles (figure 1).

Dysregulated transient receptor potential (TRP) channel activity has been associated to IBS and constipation, and peppermint oil (which contains the TRPM8 activator menthol as its biologically active ingredient) is reported to induce symptoms relief in patients with IBS and to exert spasmolytic effects and inhibition of GI contractility.[5–7] *TRPM8* SNP associations with slower colonic transit, constipation and IBS may thus result from allele-specific effects on *TRPM8* function (due to coding variants) and/or expression (due to regulatory variants) in the GI tract. In the meta-analysis, strongest association and IBS-C/M risk effects were observed for two SNPs (rs10166942 and rs2362290; table 1) mapping in the promoter region of the gene where transcriptional regulation takes place. These markers are computationally predicted to alter the transcription factor (TF) binding at the corresponding DNA sites and, in addition to expected transcription-related activities, pathway analysis of this TF pool yielded 'abnormal hepatobiliary system' as the top scoring mammalian phenotype (see online supplementary material methods and table S2). Additional pathway analyses of *TRPM8* coexpressed genes (see online supplementary material methods), returned 'bile secretion', 'bile acid and bile salt transport' and 'bile acid metabolic process' as the main biological processes (see online supplementary table S3). These are potentially important observations, since the mechanisms regulating *TRPM8* expression are poorly characterised, and alterations of bile acid synthesis and metabolism are implicated as causative mechanisms linked to constipation and IBS.[8]

In summary, we identify *TRPM8* polymorphisms that associate with slower colonic transit rates and increased risk of IBS with constipation (IBS-C and IBS-M). These results may contribute to identifying subsets of patients with IBS for improved therapeutic precision, and provide novel opportunities for mechanistic investigation of IBS symptom generation.

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