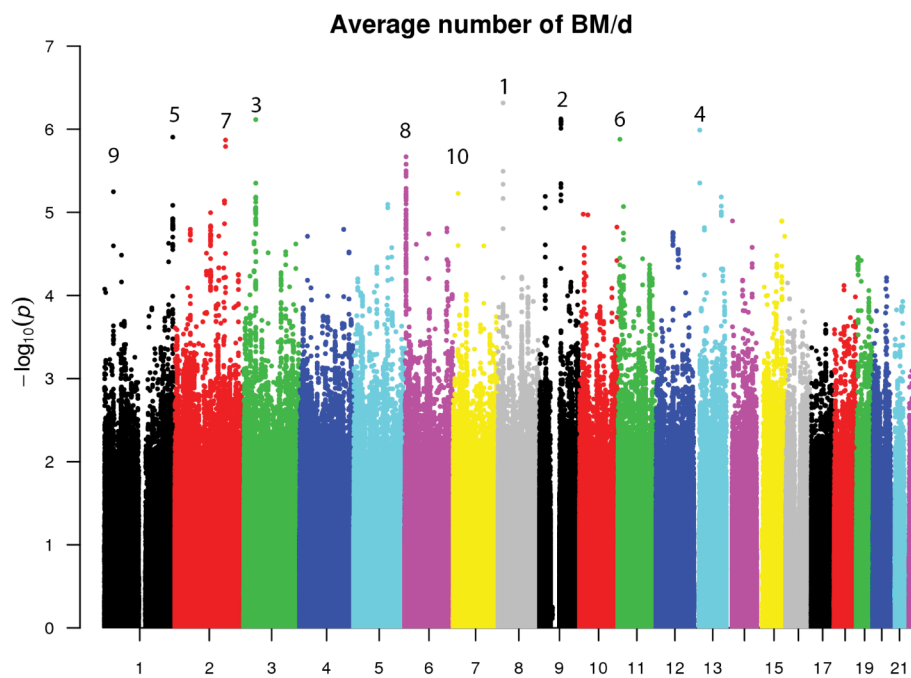


A GWAS meta-analysis suggests roles for xenobiotic metabolism and ion channel activity in the biology of stool frequency

Stool consistency and frequency patterns are complex traits that are often altered in GI disease, and recent studies published in *Gut* highlight the importance of stool frequency in relation to gut microbiota composition and the efficacy of pharmacological and dietary treatments in IBS.^{1–3}

Despite reported heritability in invertebrates⁴ and similar evidence from open-field defaecation models in rats,⁵ the genetics of stool frequency has not been explored in humans. We undertook a genome-wide association study (GWAS) in two well-characterised population-based cohorts with genotype and defaecation data available: LifeLines-Deep (LLD) from the Netherlands (N=1546; 58% females; mean age 44 years (range 18–86)) and PopCol (PC) from Sweden (N=284; 60% females; mean age 54 years (range 22–71)).^{6,7} The average number of bowel movements per day (BM/d) was extracted from daily records kept by both populations and did not differ between cohorts (LLD=1.39 ±0.64SD; PC=1.42±0.74SD). Available CytoChip+ImmunoChip (LLD) and HumanOmniExpressExome (PC) Illumina single-nucleotide polymorphism (SNP) genotype data were imputed using IMPUTE2 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html) with the Genome of the Netherlands (<http://www.nlgenome.nl/>) as reference. SNPs were filtered on minor allele frequency >0.05 and Hardy-Weinberg equilibrium $p > 1E-04$, samples were filtered on info score ≥ 0.8 and population outliers were excluded using principal component analysis. In total, high-quality genotype data for 5 390 800 common SNPs and BM/d information were obtained for 1022 LLD and 259 PC individuals. Genotype-BM/d association tests were performed in SNPTTEST (https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html) using logistic regression under an additive model correcting for age and sex, followed by a fixed-effect model meta-analysis with META (https://mathgen.stats.ox.ac.uk/genetics_software/meta/meta.html). Summary statistics for the top-10 loci from the meta-analysis and the corresponding effect of associated alleles on the frequency (increased/decreased) of defaecation are given in [figure 1](#).

Although none of these associations achieved genome-wide significance



Chromosome
Meta-analysis LLD-PC, Age and gender corrected, MAF > 0.05

#	Lead SNP	Chr	BP	SNP	Assessed allele	P-value	Beta	se	Nearest gene	Other genes in 250kb region (protein coding)
1	rs62498365	8	17622887	C/T	T	4.8E-07	-0.38	0.08	MTUS1	SLC7A2, PDGFRL, FGL1, PCM1
2	rs13959	9	75545882	G/A	A	7.5E-07	0.20	0.04	ALDH1A1	ANXA1, TMC1
3	rs735320	3	42915878	C/T	T	7.7E-07	-0.27	0.05	CYP8B1	HHATL, NKTR, ZBTB47, CCDC13, HIGD1A, KLHL40, ZNF662, FAM198A, ACKR2, KRBOX1, POMGNT2
4	rs9550650	13	21035343	A/G	G	1.0E-06	-0.27	0.06	CRYL1	GJB6, IL17D, IFT88
5	rs9782914	1	241892235	T/C	C	1.2E-06	0.29	0.06	WDR64	FH, CHML, EXO1, KMO, OPN3
6	rs4090286	11	7644539	G/C	C	1.3E-06	0.30	0.06	PPFIBP2	CYB5R2, OR5P2, OR5P3, OLFML1, SYT9
7	rs115304436	2	178944137	G/A	A	1.3E-06	0.40	0.08	PDE11A	RBM45, OSBPL6
8	rs408709	6	4568772	C/T	T	2.1E-06	0.24	0.05	CDYL	
9	rs2377852	1	31901133	C/T	T	5.6E-06	-0.27	0.06	SERINC2	FABP3, AC114494.1, NKAIN1, SNRNP40, ZC-CHC17, TINAGL1, HCRTR1, PEF1, COL16A1
10	rs1979097	7	17582940	C/G	G	5.9E-06	0.19	0.04	AHR	SNX13

Figure 1 Manhattan plot of the results from the meta-analysis of LifeLines-Deep (LLD) and PopCol (PC) genome-wide association studies. Single-nucleotide polymorphisms (SNPs) which are sorted according to their genomic positions are displayed on the X-axis, and the negative logarithm of the association p value for each SNP after meta-analysis is displayed on the Y-axis; each dot represents a SNP with a certain p value. The top-10 loci are indicated by numbers. Per locus, the statistics of the lead SNPs are shown, including the positions in the genome, the nearest genes and the genes in a 250 kb window around the lead SNPs. The effect of the assessed allele at each locus is indicated by beta; negative betas mean negative effect on the average number of bowel movements per day (BM/d) (decreased number of stool passes) and positive betas mean positive effect on the average number of BM/d (increased number of stool passes). Beta, direction of association; BP, base pair position; Chr, chromosome; SE, SE of the beta.

(possibly due to limited sample size), we found excellent functional candidates mapping to these regions. For instance, the second strongest signal included the *ALDH1A1* gene, which belongs to the family of aldehyde dehydrogenases, and another member of this family (*ALDH1A1L1*) has been shown to affect human gut microbiota composition.⁸ Moreover, Gene Network coexpression analysis (<http://www.genenetwork.nl/genenetwork/>) indicated a role for *ALDH1A1* in the cytochrome P450 metabolism of drugs and xenobiotics, and other genes in this pathway also map to top BM/d GWAS loci: the rs735320 signal comes from SNPs in the *CYP8B1* gene, which belongs to the cytochrome P450

family; the rs4090286 locus contains *CYB5R2* (cytochrome B5 reductase), which is involved in cholesterol biosynthesis, fatty acid desaturation and elongation; and the rs1979097 locus contains *AHR* (ligand-activated aryl hydrocarbon receptor), which is a transcription factor modulating gene expression along the cytochrome P450 pathway. The genetic implication of xenobiotic and P450 metabolic pathways is not unexpected, given the interactions linking diet, gut microbiome and pharmaceutical compounds to known effects on human defecation patterns, but was not reported previously.

A broader pathway analysis (<http://129.125.135.180:8080/GeneNetwork/pathway.html>), including genes from all 53 loci with $p < 5E-05$, identified the *sodium channel complex* and *voltage-gated sodium channel activity* as the most enriched pathways in Gene Ontology (GO) terms for *cellular component* and *molecular function*, respectively (table 1). This is remarkable, since genetic defects in the voltage-gated channel *SCN5A* have been found in a subset of patients with IBS, and normal stool frequency was restored in a severely constipated *SCN5A* mutant carrier treated with mexiletine, a drug able to rescue *SCN5A* expression.⁹

In conclusion, we report the first GWAS of stool frequency in two harmonised population-based cohorts from the Netherlands and Sweden and highlight

Table 1 Pathway analysis of GWAS meta-analysis results for the average number of BM/d

Top associated GO term	p Value
Cellular component	
Sodium channel complex	6E-07
Sarcolemma	2E-05
Voltage-gated sodium channel complex	2E-05
Ion channel complex	6E-05
Molecular function	
Voltage-gated sodium channel activity	2E-05
Peptidase regulator activity	3E-05
Substrate-specific channel activity	4E-05
Ion channel activity	4E-05
Passive transmembrane transporter activity	7E-05
Channel activity	7E-05
Endopeptidase inhibitor activity	8E-05

Only GO pathways with $p < 1E-04$ are reported. BM/d, bowel movements per day; GO, Gene Ontology; GWAS, genome-wide association study.

plausible candidate genes and biological pathways. Although we are not aware of similar datasets in which our findings may be replicated, the growing interest in this research area warrants larger studies to reach unequivocal conclusions.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The LifeLines-DEEP study was approved by the ethics committee of the University Medical Center Groningen, the Netherlands (document no. METC UMCG LLDEEP: M12.113965). The PopCol study was approved by Karolinska Institutet's ethics committee, Stockholm, Sweden (dnr 394/01). All participants signed an informed consent form prior to study enrolment.

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REFERENCES

- Hadizadeh F, Walter S, Belheouane M, *et al.* Stool frequency is associated with gut microbiota composition. *Gut* 2017;**66**:559–60.
- Tack J, Schumacher K, Tonini G, *et al.* The neurokinin-2 receptor antagonist ibodutant improves overall symptoms, abdominal pain and stool pattern in female patients in a phase II study of diarrhoea-predominant IBS. *Gut* Published Online First: 15 Apr 2016. doi:10.1136/gutjnl-2015-310683
- McIntosh K, Reed DE, Schneider T, *et al.* FODMAPs alter symptoms and the metabolome of patients with IBS: a randomized controlled trial. *Gut* Published Online First: 14 Mar 2016. doi:10.1136/gutjnl-2015-311339
- Branicky R, Hekimi S. What keeps *C. elegans* regular: the genetics of defecation. *Trends Genet* 2006;**22**:571–9.
- Blizard DA, Adams N. The Maudsley reactive and nonreactive strains: a new perspective. *Behav Genet* 2002;**32**:277–99.
- Tigchelaar EF, Zernakova 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.
- Walter SA, Kjellström L, Nyhlin H, *et al.* Assessment of normal bowel habits in the general adult population: the Popcol study. *Scand J Gastroenterol* 2010;**45**:556–66.
- Goodrich JK, Davenport ER, Beaumont M, *et al.* Genetic determinants of the Gut Microbiome in UK Twins. *Cell Host Microbe* 2016;**19**:731–43.
- Beyder A, Mazzone A, Stregge PR, *et al.* Loss-of-function of the voltage-gated sodium channel NaV1.5 (channelopathies) in patients with irritable bowel syndrome. *Gastroenterology* 2014;**146**:1659–68.