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 infoscore ≥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 SNPTEST (https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snpst.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
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 (ALDH1AL1) 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 defaecation patterns, but was not reported previously.

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

![Figure 1](PostScript.png)

**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.
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