Effect of host genetics on the gut microbiome in 7,738 participants of the Dutch Microbiome Project

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Abstract

Host genetics are known to influence the gut microbiome, yet their role remains poorly understood. To robustly characterize these effects, we performed a genome-wide association study of 207 taxa and 205 pathways representing microbial composition and function within the Dutch Microbiome Project, a population cohort of 7,738 individuals from the northern Netherlands. Two robust, study-wide significant ($p<1.89\times10^{-10}$) signals near the *LCT* and *ABO* genes were found to be associated with multiple microbial taxa and pathways and were replicated in two independent cohorts. The *LCT* locus associations seemed modulated by lactose intake, while those at *ABO* could be explained by participant secretor status determined by their *FUT2* genotype. Twenty-two other loci showed suggestive evidence ($p<5\times10^{-8}$) of association with microbial taxa and pathways. At a more lenient threshold, the number of loci we identified strongly correlated with trait heritability, suggesting that much larger sample sizes are needed to elucidate the remaining effects of host genetics on the gut microbiome.

Introduction

The human intestinal microbial community contains trillions of microorganisms that play an important role in maintaining normal gut function and immune homeostasis[1]. Emerging evidence has shown that alterations in gut microbial composition are associated with the pathogenesis of many human diseases, including gastrointestinal disorders, metabolic syndrome, cardiovascular diseases and other conditions[2,3].

The gut microbiome is influenced by many factors, such as environmental factors including diet and medication usage[4,5], but also by host genetics. Heritability studies in twins and families have highlighted that human genetics contributes to gut microbial variation, showing heritability estimates ranging from 1.9% to 8.1%[6,7]. This observation drove the first efforts to identify genomic loci that influence gut microbiota through genome-wide association studies (GWAS). These early gut microbiome GWAS identified several microbial quantitative trait loci (mbQTL) located in genes related to the intestinal mucosal barrier, immune response and drug and food metabolism[8–11]. However, the reproducibility of these findings has been limited by differences in data processing methodologies, modest sample sizes and strong environmental effects, which, taken together, limit the detection of robust host genetic associations[12]. A recent large-scale genome-wide meta-analysis of 24 cohorts replicated the association between *Bifidobacterium* abundance and the lactase (*LCT*) gene locus[13], which had previously only been reported in single-cohort studies[6,14]. Other suggestive mbQTLs identified in this broad meta-analysis were proportional to heritability estimates from independent twin studies, indicating that additional loci found at lenient levels of significance are likely to be real but larger sample sizes are needed to reach sufficient statistical power[13]. Nonetheless, meta-analyses of mbQTL studies still lack resolution due to the high levels of heterogeneity between cohorts, which can reduce power in meta-analysis. On top of this, many existing cohorts rely on 16S rRNA measurements, which do not allow for bacterial identification at species-level resolution or for identification of bacterial pathway abundances. Indeed, measuring both species- and pathway-level abundances is essential for a further understanding of the function of an individual's microbiome. Two closely related bacterial species can have very different functions in the gut microbiome, yet pathways may be shared across distant microbial species and have roughly the same biological effect[15]. mbQTL studies using shotgun metagenomic sequencing in large population cohorts are therefore needed to overcome the high variability in microbiome definition and reveal more robust and refined associations.

To enable a broader and deeper understanding of host microbiota interactions, we used shotgun metagenomic sequencing on feces from 7,738 individuals of the Dutch Microbiome Project (DMP)[16] and matched their imputed genotypes to differences in taxa and pathway abundances. By comparing our results with summary statistics from other independent studies[13,17,18], we identify novel host microbiota interactions. Furthermore, we explore the impact of potential confounding factors in modulating these genetic effects and identify potential diet-dependent host microbiota interactions. We further assess the potential causal relationships between the gut microbiome and dietary habits, biomarkers and disease using Mendelian randomization. Finally, we carry out a power analysis that shows how microbiome studies, even at the current sample size, are not well powered to reveal the complex genetic architecture by which host genetics can regulate the gut microbiome.

Results

Genome-wide association identifies known and novel associations with several bacterial taxonomies and bacterial pathways

We investigated 5.5 million common (minor allele frequency (MAF) > 0.05) genetic variants on all autosomes and the X chromosome, using linear mixed models[19] to test their association and following an additive genetic model, with 207 taxa and 205 bacterial pathways in 7,738 individuals from the DMP cohort (**Methods, Supplementary Table 1**)[19]. Our quality control steps did not detect evidence for test statistic inflation (median genomic lambda 1.002 (range 0.75–1.03) for taxa and 1.004 (range 0.87–1.04) for pathways). We identified 37 SNP trait associations at 24 independent loci at a genome-wide *p*-value threshold of $5x10^{-8}$ (**Figure 1, Supplementary Table 2**). Genetic variants at two loci passed the more stringent study-wide threshold of $1.89x10^{-10}$ that accounts for the number of independent tests performed (**Methods**). The other 22 loci were associated at *p*-values between $5x10^{-8}$ and $1.89x10^{-10}$, with four associated to taxa and 18 to pathways. We refer to these 22 loci as "suggestive" and discuss them below.

The strongest signal was seen for rs182549 located in an intron of *MCM6*, a perfect proxy of rs4988235 (r^2 = 1, 1000 Genome European populations), one of the variants known to regulate the *LCT* gene and responsible for lactase persistence in adults (ClinVar accession RCV000008124). The T allele of rs182549, which confers lactase persistence through a dominant model of inheritance, was found to be associated with decreased abundances of the species *Bifidobacterium adolescentis* ($p = 7.6x10^{-14}$) and *Bifidobacterium longum* ($p = 3.2x10^{-08}$), as well as decreased abundances of higher-level taxa (phyla Actinobacteria $p = 2.3x10^{-13}$; class Actinobacteria, $p = 2.3x10^{-13}$; order Bifidobacteriales, $p = 5.9x10^{-15}$; family Bifidobacteriaceae, $p = 5.86x10^{-15}$; genus *Bifidobacterium*, $p = 8.59x10^{-15}$). Associations at this locus were also seen for other taxa of the same genus, but at lower levels of significance (*Bifidobacterium catenulatum*, $p = 3.9x10^{-5}$), and for species of the *Collinsella* genus (**Supplementary Figure 1**). The genetic association at the *LCT* locus has been previously described, albeit only at the genus level, in Dutch, UK and US cohorts[6,8,14], as well as in a recent large-scale meta-analysis[13]. The presence of the *LCT* association across several taxa suggests that this locus has a wide-ranging effect on microbiome composition.

The second locus that passed study-wide significance consists of genetic variants near the *ABO* gene. *ABO* encodes the BGAT protein, a histo-blood group ABO system transferase.

Associations found at this locus include species *B. bifidum* (rs8176645, $p = 5.5x10^{-15}$) and *Collinsella aerofaciens* (rs550057, $p = 2.0x10^{-8}$, $r^2 = 0.59$ with rs8176645 in 1000 Genomes Europeans) and higher order taxa (rs550057, genus *Collinsella,* $p = 9.3x10^{-11}$; family Coriobacteriaceae, $p = 3.01x10^{-9}$; order Coriobacteriales, $p = 3.03x10^{-9}$) (**Supplementary Figure 1**). Interestingly, the metabolic pathway representing the bacterial degradation of lactose and galactose was also associated to the *ABO* locus (Metacyc ID: "*LACTOSECAT-PWY: lactose and galactose degradation I*", rs507666, $p = 5.38x10^{-15}$). Associations of this locus with the genus *Collinsella* and the metabolic pathway *LACTOSECAT-PWY* have been recently described[18,20]. To our knowledge, the associations with *B. bifidum* and *C. aerofaciens* are novel.

Association at *LCT* reflects the lactase persistence inheritance model and affects multiple taxonomic levels and bacterial pathways

Given that lactose tolerance is inherited in a dominant fashion, we tested the associations found in this locus using a dominant model for the alternative allele at SNP rs182549 and thereby compared lactase persistent (LP) to lactose intolerant (LI) individuals. Indeed, all seven taxa associated to the *LCT* locus at genome-wide significance showed a stronger association signal when we used a dominant model (all associations $p < 2x10^{-27}$), with decreased taxa abundance in LP individuals (**Supplementary Table 3**). The associations seen at the family level could mostly be accounted for by species *B. adolescentis* (no significant difference in effect size, Cochran's Q p values>0.05), while lower effects were seen for species *B. longum* and *B. bifidum* (Cochran's Q p values when comparing effect sizes with those observed for *B. adolescentis*: 0.018 and 0.003). Moreover, the association with these species remained unchanged when adding *B. adolescentis* to the association models, indicating that the associations are independent and not driven by species correlation.

We further tested the other 200 taxa for this SNP and the dominant model. Intriguingly, we also observed suggestive association ($p < 1x10^{-4}$) with rs182549 for taxa that were associated to *ABO* locus in our GWAS analysis (*Collinsella* genus and species *B. bifidum* and *C. aerofaciens)* and for the species *Roseburia inulinivorans* of the family Lachnospiraceae (**Supplementary Table 3**). For all but *Roseburia inulinivorans,* there was a consistent direction of effect across the associated taxa, with increased abundance in LI compared to LP individuals (**Figure 2** and **Supplementary Figure 2**).

Finally, when comparing the abundance of bacterial pathways between LI and LP groups, we also observed a higher abundance of the *LACTOSECAT-PWY* in LI individuals (effect = +0.300

in standard deviation (SD) units, standard error (SE) = 0.49, p = 1.02x10^{-9}). This is not surprising given that in our dataset this pathway correlates mostly with class Actinobacteria and species *B. adolescentis (*Spearman correlations r_S: 0.73 and 0.69, respectively)*,* which are both associated with SNPs at the *LCT* locus.

Associations at *ABO* are dependent on secretor status

To further understand the mechanisms underlying the association signals at the *ABO* locus, we derived blood-group types based on the genotype status of three genetic variants (see **Methods**). The majority of the individuals were either type A (40%) or type O (48%), reflecting estimates from previous studies[21]. Genetic associations at this locus could be explained by differences between non-O blood type individuals and O blood type individuals. We observed higher abundances of *Collinsella* and the metabolic pathway *LACTOSECAT-PWY* in blood type A or AB individuals compared to individuals with blood type O (Wilcoxon test for *Collinsella*, blood type O vs. A: p = 2.8x10^{-9}, O vs. AB: p = 0.11; Wilcoxon test for metabolic pathway, O vs. A: p = 5x10^{-14}, O vs. AB: p = 0.038). In contrast, blood type O individuals had the highest observed abundance of *B. bifidum* compared to other blood type groups (Wilcoxon test blood type, A vs. O: p = 2.3x10^{-14}, B vs. O: p = 0.007, AB vs. O: p = 0.006) (**Figure 3**).

The histo-blood group ABO system is determined by two genes: *FUT1*, which encodes a fucosyltransferase that assemblies the H antigen, and *ABO*, which encodes a glycosyltransferase that assemblies A or B antigens by adding a different terminal sugar to the H antigen precursor. Therefore, the presence of A, B or H antigens on the surface of presenting cells determines the blood type (A, B, or O, respectively) of an individual[22]. The gene *FUT2*, encodes for a soluble form of the H antigen, that allows these antigens to be expressed on intestinal mucosa and in bodily fluids[23]. However, individuals carrying two copies of the alternative allele A at the enzyme-inactivating nonsense mutation rs601338 (OMIM 182100) located in the *FUT2* gene are unable to express blood antigens on the intestinal mucosa (non-secretors)[24]. We observed that the associations of taxa and bacterial pathways with blood types were present in secretors and absent in non-secretors (**Figure 3**). This indicates that the associations at the *ABO* locus are likely to be driven by the exposure of A/B antigens to gut bacteria. These results recapitulate recent evidence for an mbQTL association at the *ABO* locus depending on *FUT2* genotypes with the *Collinsella* genus and with another bacteria reported in a Finnish population cohort[18]. Associations of a functional variant in the *FUT2* gene to other

bacterial taxa were observed in a recent meta-analysis, but none of these bacterial taxa or pathways showed significant association for this locus in our cohort[13].

These novel associations with species levels at *ABO* are intriguing. To our knowledge, this is the first time *B. bifidum* has been associated with predicted blood type. Early genomic analyses suggest that, among *Bifidobacterium*, *B. bifidum* is particularly adapted to the human gastrointestinal mucosa due to a unique set of genes encoding for enzymes involved in the degradation and utilization of mucin, the main component of gastrointestinal mucosa[25,26]. The degradation activity of the mucosa, where antigens are secreted, could explain the association with differences in *B. bifidum* abundance at *ABO* modulated by *FUT2*. This mechanism has been proposed for the association with *Collinsella* genus[18], and it is likely to also apply to the novel associations we identified with *C. areofaciens* and LACTOSECAT-PWY pathway abundances.

Genetic associations may be modulated by diet

Gut microbiome composition and function is known to be affected by several factors including sex, body-mass index (BMI), diet and medication usage[4]. None of our genome-wide significant associations were attenuated when including BMI, medication usage, stool frequency or stool consistency as covariates (**Methods**, all comparisons, Cochran's Q for difference in effect size $p > 0.05$), indicating that the genetic associations are independent and not confounded by these factors (**Supplementary Table 4a**). Furthermore, we did not find any sex-specific associations for the 37 SNP□trait associations, although five associations did exhibit a smaller genetic effect in females compared to males (**Supplementary Table 4b**).

We also investigated the effect of diet at the *LCT* and *ABO* loci, taking into account the dominant inheritance model at *LCT* and the observed dependence on secretor status at *ABO*. Based on 21 dietary factors derived from a food questionnaire recorded 4 years prior microbiome collection, we considered dietary factors previously associated (FDR < 0.05) with microbial taxa and pathway abundances that show genome-wide association signals in the *ABO* and *LCT* loci (see **Methods**)[16]. In an analysis that included age, sex, genetic and dietary factors, the dietary factors did not significantly attenuate the effect of the genetic components, suggesting that diet is not a source of bias in these genetic associations (**Supplementary Table 5**). Nonetheless, diet remained an important factor after correction for genetic factors. Four taxa and one pathway associated to *LCT* and *ABO* SNPs were statistically associated with at least one dietary factor ($p < 0.05$) (**Supplementary Table 5**), with a maximum of 16 factors found for *B. longum*. We further tested these associated dietary (44 diet□microbiome pairs) factors for

interaction with genetics and detected evidence for a gene□diet interaction for only one bacteria at the *LCT* locus. Specifically, we observed an increased abundance of the Bifidobacteriaceae family in LI individuals who consumed larger amounts of lactose or dairy (interaction term p = 0.03) (**Figure 3, Supplementary Table 6**), a finding that is consistent with previous reports[8,18]. In contrast, there was no evidence for interaction with diet at the *ABO* locus for the *B. bifidum* species, the *Collinsella* genus and the bacterial pathway *LACTOSECAT-PWY* (interaction term $p > 0.05$) (**Supplementary Table 6)**. This could be attributable to the limited accuracy of our diet scores, information that was recorded 4 years prior to microbiome collection. Interaction between fiber intake and genetic variants at this locus, when it is associated with *Collinsella* genus, have been reported in other populations[18].

Association signals reveal a polygenic architecture of microbiome taxa and pathways and replicate in other independent cohorts

Apart from the study-wide significant associations at the *LCT* and *ABO* gene regions, 22 other loci showed suggestive association at $p < 5\text{x}10^{-8}$ (**Supplementary Table 2**), none of which were reported previously. The majority (18) were associated with bacterial pathways that could not have been directly quantified in studies using 16S rRNA data, the methodology predominantly used in microbiome genetic studies to date. These associations included several genomic loci located in genes associated with human immune or metabolic disorders and mainly affected microbial amino acids (5), vitamin (5), nucleotide (4), TCA cycle (2), carbohydrate (1) and enterobactin toxin (1) pathways. These SNP□pathway associations provide insights into potential relationships between host phenotypes and gut microbial metabolic functions. For instance, SNP rs9927590 in 16q23.1 (in the *WWOX* gene), which has been reported to be involved in stenosis of Crohn's disease[27,28], was associated with a metabolic pathway involved in short chain fatty acid (SCFA) production (*PWY-5088*, $p = 9.2\text{x}10^{-9}$). SCFAs have been shown to boost intestinal barrier functions, reduce local inflammation and decrease the risk of immune and metabolic diseases, including type 1 and type 2 diabetes[17,29]. At another locus, SNP rs11242123 (5q31.1) was associated with a decreased abundance of nucleotide biosynthesis (*PWY-7209*, $p = 4.89\text{x}10^{-8}$) and was previously reported to affect expression of the nearby genes *IL4* and *KIF3A*, two genes involved in asthma onset[30,31].

Interestingly, two suggestive genome-wide associations point towards a role in Vitamin B9 biosynthesis. A variant (rs2714053) in 11q24.1 (in the *GRAMD1B* gene) was associated with lower abundance of bacterial genes involved in methyl diphosphate biosynthesis (*PWY-6147*, p = $4.58\text{x}10^{-8}$), and this gene was previously linked to ulcerative colitis in a Polish cohort[32].

Notably, methyl diphosphate is a precursor of folate (Vitamin B9) that is required for the survival of regulatory T cells in the human intestine[33,34]. In addition, another SNP, rs17824268 (14q23.2) was found to be associated with decreased abundance of folate (PWY-6612, $p = 2.63 \times 10^{-8}$). This SNP was reported to be associated with human blood HDL, LDL and a variety of metabolites[35,36]. Therefore, these two SNP-pathway associations point to a potential dysregulation of the host immune and metabolic system through a decrease of folate production from the gut microbiota.

In other loci, associations also point to immune and metabolic function. For example, two SNPs, rs12137024 (1p21.3, close to gene ALG14, p = 3.53x10-8) and rs78778914 (1q21.2, close to gene *RPRD2*, $p = 4.34 \times 10^{-8}$), were associated with the L-histidine processing pathway. L-histidine is usually catabolized by microbiota into glutamate, which is considered as an "immunonutrient" serving as major fuel for immune cells including lymphocytes and macrophages [37,38]. Intriguingly, the gene *ALG14* has been related to pneumonia [39], while *RPRD2* has been associated with immune diseases like asthma. Furthermore, another example is the association of SNP rs1584586 (3q25.1), near the type 2 diabetes associated gene *TSC22D2*[40], with the purine processing pathway (*PWY-841*, $p = 3.47 \times 10^{-8}$) and of SNP rs59657730 (6p12.3) within metabolic health related gene *RCAN2*, with microbial glycogen degradation (GLYCOCAT.PWY, p = 3.50x10-8) [39]support a role for host–microbiome interaction in regulating metabolic function. Four additional suggestive associations for taxa were identified, including signals in 8p22 (in the *SGCZ* gene related to diabetes)[41] and in Xp21.1 (in the *DMD* gene), although little is known about the function of the associated species.

We sought to replicate our genome-wide significant signals using summary statistics from other independent cohorts in which microbiome data was characterized using either 16S rRNA (the MiBioGen study)[13] or metagenomic sequencing (the LL-DEEP cohort)[17]. In the MiBioGen study, a meta-analysis of 24 cohorts comprising up to 18,340 individuals, the 16S rRNA measurements do not allow for the evaluation of the abundance of bacterial species and pathways and the X chromosome was not analyzed. Consequently, only 10 of our 18 SNP taxa pairs could be tested in MiBioGen and no pathways were testable. In LL-DEEP, a genome-wide microbiome association study on 952 individuals, we extracted information for the majority of the SNP taxa pairs (14/18) and SNP-pathway associations (18/19). Unfortunately, the power to replicate the associations in LL-DEEP was limited due to the small sample size. In both studies, we observed significant replication of the study-wide significant loci, *LCT* and *ABO,* using a conservative Bonferroni threshold of $p < 0.0015$ (equivalent to 32 SNP-trait pairs tested). All

seven taxa associated with SNPs near *LCT* were replicated with consistent allelic effect directions (all $p < 3.7\text{x}10^{-6}$). For the *ABO* locus, we found significant replication for the *Collinsella* genus ($p < 2\text{x}10^{-5}$ in MiBioGen) and replication at only nominal significance for the metabolic pathway *LACTOSECAT-PWY* and *B. bifidum* species ($p < 0.05$ in LL-DEEP) (**Supplementary Table 7** and **Supplementary Table 8**). The association at *ABO* with *B. bifidum* has not been reported previously and it does not reach the multiple-testing adjusted threshold for replication. Therefore, while the consistent direction of effects is encouraging, we cannot exclude the possibility for this signal to be a false positive. None of the other SNP taxa or pathway pairs were replicated in MiBioGen or LL-DEEP. Interestingly, another independent SNP in the *COL23A1* gene (rs11958296; $r^2 = 0.1$ with rs10447306 from our study) shows association in MiBioGen to the abundance of the same taxa: family Rikenellaceae ($p = 2.4\text{x}10^{-5}$) and genus *Alistipes* ($p = 9.3\text{x}10^{-6}$).

To explore if the association signals at lower levels of significance are enriched in heritable bacteria, which would indicate if it were possible to detect more genome-wide significant mbQTLs by further increasing the sample size, we investigated the correlation of taxa and pathway heritability estimations from family-based analysis with the number of suggestively associated loci for each taxon and pathway. Here we observed a positive and significant correlation for both taxonomic ($r_S = 0.248$, $p = 3.1\text{x}10^{-4}$) and pathway ($r_S = 0.263$, $p = 1.3\text{x}10^{-4}$) heritability with the number of suggestive ($p < 1\text{x}10^{-5}$) loci identified in our GWAS (**Figure 4**). The correlation for pathways remained significant when increasing the mbQTL threshold to $p < 5\text{x}10^{-4}$ ($r_S = 0.541$, $p = 6.3\text{x}10^{-17}$) and when further removing the *LCT* and *ABO* loci from the analyses ($r_S = 0.540$, $p = 6.3\text{x}10^{-17}$).

We also evaluated if we could replicate any of the genome-wide significant signals outside the *LCT* and *ABO* loci that were reported in a recent and similarly-sized Finnish population study[18]. After extracting all associations with $p < 1\text{x}10^{-4}$ in our dataset, we identified 3 out of 451 genome-wide significant SNPs from the Finnish study using direct or proxy ($r^2 > 0.8$) information (279 of the SNPs reported in this study were not included in our dataset because their MAF was < 0.05). For one SNP, rs642387, we identified an association with consistent allelic effect for similar taxa: the minor allele at this variant (through proxy SNP rs632222) was associated with a decreased abundance of the *Desulfovibrio* genus ($p = 8.7\text{x}10^{-5}$) in our study. This corroborates the allelic effects seen in the Finnish study for the abundances of phylum Desulfobacterota A, class Desulfovibrionia, order Desulfovibrionales and family Desulfovibrionaceae. For the other

taxa, we observed consistent direction in our dataset, although the signals did not reach nominal significance (p = 0.14) (**Supplementary Table 9**).

Mendelian randomization suggests salt intake and triglycerides are controlled by microbiome abundance

To investigate the causal relationships between microbiome composition/function and complex traits, and *vice-versa*, we used publicly available summary statistics in conjunction with our mbQTL results to perform two-sample Mendelian randomization (MR) analyses (see **Methods**). We focused on 78 phenotypes representing autoimmune diseases, cardiometabolic diseases, and related risk factors, as well as food preferences (**Supplementary Table 10**), and on 37 microbiome features that were associated with at least one variant at genome-wide significance ($p < 5x10^{-8}$). None were significant at FDR < 0.05. At FDR < 0.1 (corresponding to a $p = 2.7x10^{-5}$ obtained from the inverse-variance weighted (IVW) MR test), we observed three causal relationships in the direction from microbiome to phenotypes. Two of the three are based on the same SNPs used as genetic instruments: genetic predispositions to a higher abundance of family Rikenellaceae and genus *Alistipes* are linked to lower consumption of salt ('Salt added to food' ordinal questionnaire phenotype, both traits: IVW $p = 2.4x10^{-5}$, causal effect = -0.06 SDs of salt intake for each SD increase in Rikenellaceae or *Alistipes* abundance, based on three genetic instruments located on different chromosomes) (**Supplementary Table 11**) (**Supplementary Figure 3a,b**). This correlation was not significant in the opposite direction (IVW p = 0.32 for both), suggesting that Rikenellaceae abundance may have an effect on the increased consumption of salt without being tagged by a shared causal factor. The other potential causal relationship that passed our FDR < 0.1 threshold was between the genus *Collinsella* and triglyceride levels. We found that a genetic predisposition to a one SD increase in the relative abundance of *Collinsella* was associated with a 0.12 SD decrease in triglyceride levels (p = 5.7x10-5, FDR = 0.084) **(Supplementary Table 11**) (**Supplementary Figure 3**). All of these causal estimates were consistent when using other MR tests (weighted median and MR Egger) (**Methods** and **Supplementary Table 11**). Furthermore, there was no evidence for the causal effect being affected by pleiotropy (Egger intercept p = 0.74 for Rikenellaceae and *Alistipes* and 0.755 for *Collinsella*) (**Supplementary Table 11**). The results found in the two-sample MR were very similar when we performed a polygenic risk score analysis in the UK Biobank cohort (**Supplementary Notes**).

While all these potential causal effects are intriguing and exhibit robustness to sensitivity analyses, we recognize that the limited availability of genetic variants and the high FDR threshold dictate caution in their interpretation.

Power analysis indicates that larger sample sizes are necessary to identify host genetic effects for microbial features with low prevalence

One of the complicating factors in any genome-wide association study of the gut microbiome is the variable detection rate of the pathways and taxa measured, which reduces the effective sample size and consequently statistical power. In our study, the additive effect of associated variants at *LCT* explains 0.8% of the variance for species *B. longum* and *B. adolescentis*, which are present in >80% of the samples, and this allowed us to have ~70% power to detect the association at $p < 1x10^{-10}$ (**Figure 5**). In contrast, we had no power (<5%) to detect the association for species *B. bifidum* and *B. catenulatum* at this level of significance, as they were present in only 14% and 26% of samples, respectively. For *B. bifidum*, despite it being a rare species, we were able to detect a study-wide significant association at *ABO* because the effect was substantial (2.5% of the variance explained by SNP rs8176645, power = 83%), but we were underpowered to see the association at this locus with species *C. aerofaciens*, which was present in only 45% of the samples, and for which the additive effect of *ABO* variants was smaller (0.5% variance explained) (**Figure 5**). In a power analysis, we estimated that our sample size (7,738 individuals) is underpowered to detect genetic effects for taxa that are present in <80% of samples when considering an effect size comparable to or smaller than the effect of *LCT* for *B. adolescentis.* This is striking, considering that 93% of the taxa we identified in this cohort are present in <80% of the samples (**Methods**). We estimate that to find a similar effect size for at least 20% of gut microbiome composition (approximately 20% of the taxa are present in at least 20% of the samples in our cohort) would require a cohort of ~50,000 individuals.

Discussion

We carried out the largest genome-wide association study of gut microbiome composition and function in a single population by analyzing metagenomic sequencing data in 7,738 volunteers from the northern Netherlands. We recapitulated genetic associations at two known loci, *LCT* and *ABO*, and the resolution of our metagenomic sequencing allowed us to pinpoint novel associations with species (*B. adolescentis* at *LCT*, *B. bifidum* and *C. aerofaciens* at *ABO*) and bacterial pathways (*LACTOSECAT-PWY* at *ABO*) in these loci. Furthermore, we identified

associations ($p < 5x10^{-8}$) at 22 other loci for four taxa and 18 bacterial pathways. None of these associations were affected by major confounders of the gut microbiome such as medication usage, diet and BMI. Finally, we used an MR approach to pinpoint potential causal links between gut microbiome composition, complex traits and food intake habits.

The strongest association with the gut microbiome was found at the *LCT* locus, which remains the most robust genetic association with the gut microbiome identified to date. Associations at this locus with *Bifidobacterium* have been consistently reported in studies of different ethnicities, across a range of sample sizes, and in studies using different technologies and protocols for gut microbiome characterization[6,8,13,14,42]. We also recapitulate that an increase of *Bifidobacterium* was more evident in LI individuals who were consuming milk or milk-derived products[13,43]. In addition, given that the resolution of metagenomic sequencing allows for species-level characterization of microbiome profiles, we could show that this effect was mainly attributable to the species *B. longum, B. adolescentis, B. catenulatum* and *B. bifidum*, an observation that was corroborated by two recent studies in Finnish and US Hispanic/Latino populations[18,44].

We found another study-wide association in the *ABO* gene locus with changes in abundance of several taxa and pathways. Associations with microbiome composition and blood types were observed in previous experimental studies[45,46]. Genetic associations at the *ABO* locus have also been reported previously for populations of different ethnicities and in non-human species. For example, associations with *Bacteroides* and *Faecalibacterium* were reported in a study of five German cohorts that used 16S rRNA sequencing for gut microbiome characterization[42] and with the microbial pathway of lactose and galactose degradation in a cohort of 3,432 Chinese individuals[47]. The importance of *ABO* in determining host microbiome interaction has also been recently reported in pigs[48]. A deletion at this locus that inactivates the ABO acetyl-galactosaminyl-transferase has been shown to change the porcine microbiome composition by altering intestinal N-acetyl-galactosamine concentrations and consequently reducing the abundance of *Erysipelotrichaceae* strains, which have the capacity to import and catabolize N-acetyl-galactosamine[49]. In our analysis, the strongest associations were with *B. bifidum*, which has not been reported before, and with the *Collinsella* genus, which was recently reported[18], and at genome-wide significance with *C. areofaciens*. We did not detect any evidence of an interaction with diet at this locus, although this could be due to limitations in available information as the recording of dietary information was done at different times than the stool collection. We did, however, find that associations at this locus depend on secretor status that is determined by a nonsense mutation at the *FUT2* gene[42] and thus on the host's ability to

incorporate antigens into bodily fluids that are released in the gut. Intriguingly, we observed that taxa associated with the *ABO* locus also showed evidence of association at the *LCT* locus, this association being independent of blood type (interaction pvalue >0.05 for all taxa), indicating a common, independent action of these two loci in contributing to the growth of these bacteria. The most compelling hypothesis is that the availability of sugars in the gut, via undigested lactose in LI individuals or secretion of antigens with accessible glycans in non-O blood type secretors, provides direct energy sources for most of these bacteria. This is further supported by the observation that LI individuals and non-O blood type secretors were both associated with the increased abundance of a bacterial pathway for lactose and galactose degradation. However, this mechanism would not fully explain the opposite direction of the association at *ABO* seen for *B. bifidum,* which, under this hypothesis and considering its adaptation to normal gastrointestinal mucosa (apparently independent of H-antigen secretion, as shown in **Figure 4**), would be subjected to competition in the environment, such as in the case of non-O blood secretors subjects. Of note, a similar pattern of complex association at this locus was found in a recent study of German cohorts[42], where a branch of the Bacteroides genus represented by OTU97_12, OTU99_12, and TestASV_13, showed association with an inverse relationship between their prevalence and the non-O blood type group and instead a positive relationship with prevalence of OTU97_27. While these opposite associations could be explained by antigen degradation activity of certain species and consequent environment competition for others, more studies are needed to clarify the complex mechanisms involved

We acknowledge that anachronistic diet information is a limitation of our study. While we have shown that in general the microbiome remains fairly stable in an individual after 4 year, with inter-individual differences being larger than inter-individual differences[50] , short-term changes in diet, especially those introducing drastic shifts, can perturbate both microbiome composition and function. These could not be taken in account by our analyses. Capturing these shifts would not be easy even with frequency food questionnaires recorded at the time of sample collection; ideally, real-time extensive recording in weeks preceding microbiome collection should be implemented in future biobanks.

The strongest mbQTLs we identified reside in genes under selective pressure. The *LCT* gene is a highly differentiated gene among human populations due to positive selection conferred by the lactase persistence phenotype. It has been estimated that strong selection occurred within the past 5,000–10,000 years, consistent with there being an advantage to lactase persistence and the ability to digest milk in the setting of dairy farming[51]. Variants at this locus have been linked

through GWAS to not only food habits and metabolic phenotypes, but also to immune cell populations[52]. The *ABO* locus is evolutionarily highly differentiated; it has been shown to have experienced balancing selection for the last 3 million years in many primate species[53]. Several evolutionary sources of selective pressure have been proposed, including via infections by pathogens such as malaria[54] and cholera[55]. *ABO* variants have also been linked to cardiometabolic traits, white and red blood cell levels and cytokine levels[56,57]. Therefore, host microbiome interactions are likely to be shaped by human microbe co-evolution and survival, probably through a balance between food availability for gut bacteria and enhanced immune response of the host. A better understanding of these interactions will expand our current knowledge of human evolution[58].

From this perspective, it will be crucial to compare genetic studies of the gut microbiome in diverse populations with different genetic backgrounds in order to understand the complexity of host microbe interactions. This requires community efforts to standardize the definition of taxonomies and to standardize measurement methodologies in order to facilitate comparison between cohorts. For example, in our attempt to replicate the findings from the Finnish population cohort, only a limited number of taxa could be directly matched or connected through the Genome Taxonomy Database[59].

To explore the causality of the relations of the microbiome with complex traits and food preferences, we performed bi-directional MR analysis using 56 dietary traits, 16 diseases and 5 biomarkers. At FDR < 0.1, we observed three causal relationships. Although the limited impact of genetic variants on both microbiome composition and dietary preferences requires caution when interpreting causality estimation by MR[60,61], it is intriguing to observe a causal role for microbial composition on food preferences. Here we observed that an increase in the abundance of genus *Alistipes* and its family Rikenellaceae led to decreased consumption of salt. Although multiple studies have shown that dietary changes, such as variations in salt and meat intake, have a strong effect on microbiome composition[62], it is intriguing to suggest that genetically determined variations in the microbiome might affect individual food preferences. This would be supported by bacterial genetic variations in salt tolerance[63] and by the established knowledge that the composition of gut microbiome can accurately predict the effect of food items on host metabolism[64]. There was additional evidence from our results to support a role for the microbiome in influencing food preferences. A perfect proxy of rs642387 (rs503397, r^2 = 0.99 in 1000 Genomes Europeans), which was recently reported to be associated with the family Desulfovibrionaceae and related taxa in a Finnish population and replicated in our study,

had previously been associated to bitter alcoholic beverage consumption in an independent cohort[65]. Another study found an increase in family Desulfovibrionaceae in individuals with high alcohol consumption[66], and family Desulfovibrionaceae, genus *Desulfovibrio* and other related taxa were also associated with increased consumption of alcohol in our DMP cohort[16], supporting a pleiotropic effect of this locus on both microbiome and alcohol intake. These studies, together with our findings, suggest that gut microbiota could indirectly influence an individual's food preferences by mediating the downstream effect of the consumption of different products.

Understanding the differences in microbial pathway abundance is crucial for deeply understanding the underlying function of the gut microbiome. Microbiome measurements that target single organisms or taxonomic groups may be inefficient because two strains of one species may differ in their functional capacities. It is therefore essential to evaluate not only the taxonomic but also the functional composition of the microbiome. To our knowledge, we report the largest single study to investigate host genetic effects on the human gut microbiome function using metagenomics sequencing and thereby perform genome-wide association analysis of microbial pathways. Our analyses revealed several loci associated with microbial pathways, although only one passed stringent study-wide significance. Functional pathway abundances are also an approximate indicator of metabolite expression. For example, we report an association with PWY-5088, which is involved in propionate production. Although we do not have SCFA levels in our samples for direct validation, we have measured propionate levels in feces in the LL-DEEP cohort and using summary statistics from our previous study[17], we see consistent directions of effect for the association with PWY-5088 (allele T was associated with decreased levels of propionate), but the association was not significant, perhaps due to the small sample size (N=897, p value = 0.08). It also should be noted that pathway abundance, even if not necessarily representing the expression, might represent the gene load and abundance of the bacterial taxa that are able to execute this metabolic pathway, which by itself is an important indicator for the metabolic capacity of a given gut microbiomal community. We believe more metabolite screening should be undertaken once more robust and consistent replications are detected for microbial pathways by other genetic studies.

In addition to the two study-wide significant loci, we also observed 22 loci at genome-wide significance and many more at a more lenient threshold. The correlation we observe between the heritability of microbial taxa and pathways and the number of suggestively associated loci indicates that mbQTLs with smaller effects are likely to exist. Those loci remained under the

detection limit in the current study, and the sample size would need to be increased by orders of magnitude to discover their role. In fact, gut microbiome composition is characterized by high inter-individual variation. Analysis of core microbiota in different populations indicates that only a few bacteria are present in >95% of studied individuals[5,13,16], drastically reducing the effective sample sizes for analyses. We performed power calculations taking into account the inter-individual variations in microbiome composition and concluded that a sample size comparable to our study (~8,000 participants) is only sufficient to identify associations to taxa present in >80% individuals, which comprise only 7% of all identified taxa in the cohort. For bacteria present in >20% of the samples, >50,000 participants would be necessary to identify an effect size similar to that of *LCT* and *ABO*. Much larger cohorts are therefore required to identify genetic contribution to rarer bacteria. We conclude that joint efforts that combine tens of thousands of individuals, combined with harmonized methodology to reduce technical bias, will be needed to characterize more than a few major loci, as has also been the case for genetic studies of much more heritable quantitative traits such as BMI (heritability ~40%), height (heritability ~80%) and other human phenotypes[67–69].

Methods

Cohort description

Lifelines is a multi-disciplinary prospective population-based cohort study with a unique three-generation design that is examining the health and health-related behaviors of 167,729 people living in the north of the Netherlands. Lifelines employs a broad range of investigative procedures to assess the biomedical, socio-demographic, behavioral, physical and psychological factors that contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics[70,71]. During the first follow-up visit, all participants were invited to participate in a parallel project, The Dutch Microbiome Project (DMP), on a voluntary basis. The goal of this project is to evaluate the impact of different exposures and life-styles on gut microbiota composition[16]. A subset of 10,000 Lifelines participants were included, for 8,719 of them both feces and phenotype information were collected. Of these, 8,208 were retained for downstream analysis after stringent quality control[16]. Distribution of age, gender and location within the three north provinces was similar to that observed in the total Lifelines cohort[71].

The Lifelines study was approved by the medical ethical committee from the University Medical Center Groningen (METc number: 2017/152). Additional written consents were signed by the DMP participants or legal representatives for children aged under 18.

Genome characterization

Genotyping of 38,030 Lifelines participants was carried out using the Infinium Global Screening array® (GSA) MultiEthnic Diseases version, following the manufacturer's protocols, at the Rotterdam Genotyping Center and the Department of Genetics of the University Medical Center Groningen. Here, we used available quality-controlled genotyping data imputed with Haplotype Reference Consortium (HRC) panel v1.1[72], as described elsewhere[73]. To avoid population stratification, we analyzed only European samples. We selected 43,587 genetic markers by applying linkage disequilibrium (LD) pruning on the genotyped data (sliding window of 1Mb, linkage disequilibrium $r^2<0.2$, step = 5) and used them for a Principal Component Analysis (PCA) projecting the 1000 Genomes (all populations) and GoNL cohorts a population references[74,75]. Europeans were then identified as samples clustering together with European populations according to the first two principal components or <3 standard deviations away from the most distant European samples in the reference. In total, 35 non-European participants were removed. Quality-controlled genotype information was obtained for 7,738 of the DMP participants for whom quality-controlled microbiome data and BMI were also available. Of these, 58.1% were females and had ages ranging from 8 to 84 (mean of 48.5 years). The mean BMI value was 25.58 (range from 13.10 to 63.70).

Microbiome characterization

The gut microbiome was characterized from stool samples as described in Gacesa et al[16]. In brief, stool samples were collected by participants and frozen within 15 minutes after production and then transported on dry ice to the Lifelines facility to be stored at -80°C. Microbial DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany), following the manufacturer's instructions. Samples with a total DNA yield lower than 200 ng (as determined by Qubit 4 Fluorometer) were prepared using NEBNext® Ultra™ DNA Library Prep Kit for Illumina, and samples with higher DNA yield were prepared using NEBNext® Ultra™ II DNA Library Prep Kit for Illumina®. Shotgun metagenomic sequencing was carried out using the Illumina HiSeq 2000 platform at Novogene, China. Metagenomic sequencing data was profiled following methods previously used in other cohorts, as described in[16,76]. Low quality reads

(PHRED quality ≤ 30), adapters and host sequences were removed using KneadData tools v0.5.1. Taxonomic composition was determined with MetaPhlAn2 v2.7.2 [77]. Characterization of biochemical pathways was performed with the HUMAnN2 pipeline v0.11.1[78], integrated with the UniRef90 v0.1.1 protein database[79], the ChocoPhlAn pangenome database and the DIAMOND alignment tool v0.8.22[80]. After quality control (samples with eukaryotic or viral abundance ≤ 25% and total read depth ≥ 10 million were retained), we had information on 950 microbial taxa and 559 functional pathways. For this study, we focused only on bacterial taxa and functional pathways with mean relative abundance >0.001% across all samples and present in at least 1000 of the 7,738 participants, which resulted in a list of 207 taxonomies (5 phyla, 10 classes, 13 orders, 26 families, 48 genera and 105 species) and 328 bacterial pathways. Furthermore, we removed redundant pathways by discarding one pathway among pairs that were highly correlated (r_S > 0.95), as well as pathways not previously described in bacteria that could thus be coming from sources other than bacteria, resulting in 205 pathways for genetic analyses.

Diet phenotypes definition

Dietary habits were assessed using a semi-quantitative Food Frequency Questionnaire (FFQ) designed and validated by the division of Human Nutrition of Wageningen University as described before in Siebelink et al and Gacesa et al.[16,81]. The FFQ data was collected 4 years prior to fecal sampling, and supplementary questionnaires were collected concurrent with fecal sampling, with the stability of long-term dietary habits between time points assessed as described in Gacesa et al.[16]. We analyzed the dietary factors that were previously found to be associated (FDR < 0.05) to the microbiome features in our study that had a genome-wide significant signal in the *ABO* and *LCT* loci. We also analyzed lactose intake for the species *Bifidobacterium longum* and *Bifidobacterium adolescentis*, given their association with the *LCT* region in our study. Participants with an implausible caloric intake (< 800 or > 3934 kcal/day for males, < 500 or > 2906 kcal/day for females)[82] were not included in these analyses.

GWAS analysis method

Genome-wide association analysis was performed in 7,738 European samples for 412 features (205 functional pathways and 207 microbial taxa), investigating genetic additive effects using allele dosages for 5,584,686 genetic variants with MAF > 0.05 and information score > 0.4 on the autosomes (chromosomes 1-22) and the X chromosome. We focused on the quantitative dimensions of relative bacterial and pathway abundances, treating all zero values as missing data. We used natural-log-transformed abundances and regressed these in a linear mixed model using SAIGE v.0.38[19], with age, sex and the genetic relationship matrix (GRM) among participants as covariates. We used the standard settings of SAIGE, which applies inverse-rank normalization to the traits prior to the association analyses. The GRM was built with SAIGE using a set of 54,565 SNPs selected from the total set of quality-controlled SNPs directly genotyped and filtered for allele frequency and redundancy (MAF ≥ 0.05, $r^2 < 0.2$, sliding window = 500Kb).

Definition of the study-wide significant threshold

To estimate the number of independent phenotypes assessed, we used PCA on the matrix of 412 microbiome features (207 taxonomies and 205 pathways) available for GWAS analysis to decompose variability in independent components (axes). We estimated that 264 components are needed to explain 90% of the microbiome variance. We then defined our study-wide significant *p*-value threshold by correcting the genome-wide significance threshold for this factor ($5x10^{-8}/264 = 1.89x10^{-10}$).

Association using dominant model

To evaluate association using a dominant model on SNP rs182549 at the *LCT* locus, we used best-guess genotypes and converted T/C to T/T. Association analysis was then run for all taxa as well as the *LACTOSECAT-PWY* pathway using SAIGEgds[83] and the same covariates and transformation used for the GWAS analysis.

Inference of blood groups

We estimated blood groups from genotyped and imputed data following the scheme of Ellinghaus et al.[84]. Specifically, we used the absence of the rs8176719 insertion to define blood-type allele O1, the T allele of rs41302905 to define blood-type allele O2 and the T allele of

rs8176746 to define the blood-type allele B (instead of rs8176747). Diploid individuals O1O1, O2O2 and O1O2 were considered blood type O. Diploid individuals O1B, O2B and BB were considered blood type B. Absence of the alleles mentioned above was used to define blood-type allele A. To evaluate differences across blood types, we compared the mean relative abundance of microbiome features in individuals with A, B and AB blood type to that in individuals with the O blood type using a two-sided Wilcoxon test. To evaluate the interaction with the rs601338 *FUT2* (secretor/non-secretor) locus, we grouped individuals into two groups (non-O blood type and blood type O) to distinguish production or non-production of antigens, and compared pairs using a two-sided Wilcoxon test. All analyses were done using base R v3.6.1 (https://www.R-project.org/).

Effects of potential confounders on significantly associated loci

We evaluated the robustness of genome-wide associated signals by incorporating the following potential confounders into our statistical model: medication usage, anthropometric data and stool frequency and consistency data (collection and processing was described in Gacesa et al.[16]). We analyzed the effects of the following medication groups: proton pump inhibitors (ATC A02BC, N = 130), laxatives (osmotic ATC A06AD, N = 44; volume increasing ATC A06AC, N = 77), one group of antibacterials (ATC J01, N = 24) and other anti-infectives (ATC J, N = 39). The other group of medication considered was antibiotic use in the 3 months prior to stool collection (N = 450). For each of these medications, we created dichotomous variables for all participants coded as 0 (non-user) or 1 (user). The other factors included were BMI, stool frequency and stool consistency (mean Bristol stool scale). All models also incorporated age and sex as covariates and were run only for the genome-wide-significant SNP-trait pairs (**Supplementary Table 1**) using the same software used for GWAS (SAIGE, Zhou et al.[19]). To evaluate the impact of these covariates on the genetic signals, we used Cochran's Q heterogeneity test to compare the effect size obtained by the covariate-inclusive model and the basic model (that only includes age, sex and the genetic variant). To evaluate the impact of sex, we ran the SNP-association analysis in SAIGE separately for males and females using only age as a covariate. For each genetic variant, differences in effect size in males and females were tested using Cochran's Q heterogeneity test.

Interaction analyses

We used a three-step procedure to evaluate gene-diet interactions for all the taxa and pathways associated with SNPs at the *LCT* and *ABO* loci. First, we extracted the variables

representing dietary habits that had previously shown significant association with these microbial traits at FDR<0.05[16]. For the genus *Collinsella* and pathway *LACTOSECAT-PWY*, no dietary factors were found at this FDR threshold. We therefore considered the same dietary factors associated with B. *bifidum* in the analyses, given that they showed similar patterns of genetic association. Next, we added these variables to the basic genetic model (feature ~ age + sex + genetic variant) to confirm their association at (at least) nominal significance level (p < 0.05) while accounting for the associated genetic variant(s). Finally, for the dietary variables showing nominal significance, we evaluated the interaction with the genetic variant(s) by including an interaction term into the association model. For the *LCT* locus, we considered a binary variable to distinguish two groups of genotypes at SNP rs182549 (C/C vs. C/T and T/T) according to the dominant inheritance model at this locus. For the *ABO* locus, we used a binary definition of blood type (blood type O vs A/B/AB) and also considered the effect of the rs601338 genotype in the *FUT2* gene (defining secretor/non-secretor individuals). All microbiome features were inverse-rank normalized before analyses, and age and sex were added as covariates as in the main GWAS analysis. All models were fit using the lm() function from base R v3.6.1, other statistical tests were as implemented in packages *rstatix* v0.5.0 and *ggpubr* v0.3.0 and package *RNOmni* v0.7.1 was used for the inverse-rank normalization.

Replication in other cohorts and data sets

We looked for replication of our results using summary statistics from two independent studies: a genome-wide meta-analysis of 16S rRNA data from 24 cohorts (the MiBioGen consortium) and a genome-wide study on metagenomics data in the LL-DEEP cohort, another subset of the Lifelines cohort with data generated 4 years before the DMP and in which 255 participants were also later enrolled in DAG3[13,17]. In this study, a different DNA isolation procedure (AllPrep Kit) was used and taxonomies were defined using the Bracken pipeline, which may explain why C. areofaciens was not identified. In the MiBioGen study, we could not look at SNPs associated with species or pathways, as these microbiome features cannot be defined using 16S data, or at SNPs on the X chromosome, as they were not analyzed in this study. In the second study, we searched for the exact same taxonomy or pathway, but similarly to MiBioGen, X chromosomal variants were not tested and some taxonomies were not defined due to the differences in metagenomic data processing pipelines.

Next to the replication of our findings, we also evaluated whether the genome-wide signals reported in a recent genome-wide study of microbiome taxa from a Finnish cohort were replicable in our data[18]. We searched for all SNPs outside the *LCT* and *ABO* loci or any proxy (r^2

> 0.8) in our dataset and selected all SNP taxonomy pairs that showed a $p < 1x10^{-4}$ with at least one taxonomy in our cohort (**Supplementary Table 9**). We then looked at these associated taxa in the respective cohorts and compared them visually and with the aid of the Genome Taxonomy Database (https://gtdb.ecogenomic.org/) to determine if they were the same bacterial taxa or taxa from the same taxonomic branch.

Correlation between heritability estimates and the number of associated loci

To analyze the correlation between family-based heritability and the number of suggestive mbQTLs, we used narrow-sense heritability estimates for taxa and pathways that accounted for household environment sharing and previously derived for this cohort[16]. We then calculated the number of independent mbQTLs per microbial trait by performing LD pruning ($r^2 < 0.1$ in our data set, window size 1Mb using Plink v1.9[85]) for all SNPs at the three different thresholds: $p < 5x10^{-4}$, $< 1x10^{-4}$ and $< 5x10^{-5}$. The association of heritability and the number of mbQTLs was calculated in R v.4.0.3 using a weighted Spearman correlation from the `wCorr` v1.9.1 package, with each taxon or pathway treated as a data point. The weights used in calculating the correlation were inversely proportional to the Z scores calculated from heritability pvalue estimates. The regression lines in **Figure 4** were fit using the *loess* function (locally estimated scatterplot smoothing, base R package v4.0.3) with span and degree parameters set to 1.

Mendelian randomization analysis

To evaluate potential causal relationships between the gut microbiome and other common traits, we performed Mendelian randomization (MR) analyses that combined the summary statistics of the microbiome with publicly available summary statistics on food preferences, autoimmune and cardiovascular diseases and other cardio-metabolic traits. We analyzed the 37 microbiome features (pathways and taxa) with at least one variant passing the $p < 5x10^{-8}$ threshold (**Supplementary Table 2**) and combined these with 78 publicly available summary statistic datasets retrieved using the IEU GWAS DATABASE[86].

We performed a bi-directional MR analysis, first testing if microbiome traits causally affect a phenotype and then testing if phenotypes can causally affect the microbiome traits. For each comparison, we intersected the microbiome variants (MAF > 0.05) by rsID, position and alleles with the publicly available summary statistic variants. We then selected instruments using the 'clump_data()' function of the TwoSampleMR package (v0.5.5)[87]. The publicly available summary statistics were clumped using a *p*-value threshold of $< 5x10^{-8}$ and otherwise standard

settings (r^2 < 0.001, 10mb window size). Due to the limited statistical significance of the microbiome traits, we performed *p*-value clumping at a less stringent $p < 5x10^{-6}$ threshold. If fewer than three variants were clumped, we removed the trait combination from analysis.

The MR analysis was done using the TwoSampleMR v0.5.5 package. We first selected trait combinations that passed the Benjamini-Hochberg FDR threshold of 0.1 ($p = 2.805x10^{-5}$) in the inverse-variance weighting (IVW) test, resulting in one suggestively causal trait combination. We further checked that the significant trait combinations were unlikely to be driven by pleiotropy based on two criteria: i) the Egger regression intercept was nominally significant (indicating the presence of horizontal pleiotropy) and ii) the weighted median results were not nominally significant (indicating that no single variant influences the result)[88,89].

Power analysis

We calculated the variance explained at our loci using the formula described in Teslovich et al.[90], which takes into account MAF, effect size, standard error and sample size. We then performed a power analysis based on a linear model of association, considering different genetic effect sizes (variance explained) and sample sizes (N) <https://genome.sph.umich.edu/wiki/Power_Calculations:_Quantitative_Traits>. We performed a sample size calculation by doing a grid search in the sample size sequence: [1000, 1050, … , 50000] and kept the lowest sample size that had power > 80%.

Data availability

Raw sequencing microbiome data is available at EGA (accession number EGAS00001005027**)**. Genotyping data and participant metadata are not publicly available to protect participants' privacy and research agreements in the informed consent. The data can be accessed by all bona-fide researchers with a scientific proposal by contacting the Lifelines Biobank (instructions at: https://www.lifelines.nl/researcher). The full summary statistics are available for download at https://dutchmicrobiomeproject.molgeniscloud.org. The processed microbiome data (taxonomy and pathway abundance per individual) can also be downloaded after filling in a request form available at the same website and after signing a data access agreement. The authors declare that all other data supporting the findings of this study are available within the paper and its **Supplementary Information** files.

LifeLines Cohort Study - group authors genetics

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Author Contributions

ELM, AK and SH performed genetic analyses.

AvdG performed Mendelian randomization and power analyses.

AK, SH, LC, AVV and RG processed microbiome data.

SAS, TS, VC, MAYK, LAB and MBG processed samples meta-data.

PBTN and MAS provided resources on the HP computing cluster and assisted with data management.

HJMH, CW, JF, RKW and AZ provided funding resources and designed the DMP.

AZ and SS supervised the study.

ELM, AK, AvdG, SH, SAS, AZ and SS drafted the manuscript.

All authors were involved in data interpretation and provided critical input to the manuscript draft.

Competing Interests statement

The authors declare no conflicts of interest.

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Figures

Figure 1. Genome-wide association scan results.

Manhattan plot of host genomic associations to bacterial taxa and bacterial pathway abundances with at least one genome-wide significant association ($p < 5x10^{-8}$). Y-axis shows the -log10 transformation of the association *p*-value observed at each tested variant. X-axis shows the genomic position of variants. The thresholds of study-wide ($p = 1.89x10^{-10}$) and genome-wide ($p = 5x10^{-8}$) significance are shown as horizontal red and blue lines, respectively. Independent SNP trait associations reaching genome-wide significance are listed in the tables and labelled on the Manhattan plot. The colors of associated hits indicate whether they represent an association with taxonomy (orange) or a bacterial pathway (blue).

Figure 2. Association at the LCT locus and interaction with lactose intake.

Comparison of f. Bifidobacteriaceae relative abundance a) between groups of Lactase Persistent (LP, rs182549 C/T or T/T) and Lactose Intolerant (LI, rs182549 C/C) participants and **b)** stratified among individuals with low or high daily lactose intake levels. Lactose intake was corrected for daily calorie consumption. Relative abundance was natural log–transformed and adjusted by age and sex. Distribution is displayed with violin plots, where each inner box represents the first and third quartiles of the distribution and the middle line represents the median. Lactose intake levels were defined as low if < first quartile and high if ≥ first quartile. Lactose intake was only available for 5,801 of the 6,809 LP samples and for 376 of the 443 LI samples. *P*-values were obtained with a two-sided Wilcoxon rank test. Species in the same family shared similar distributions, although the difference in distribution within the LI group is not significant (**Supplementary Figure 2**).

Figure 3. Association with blood types and interaction with *FUT2.*

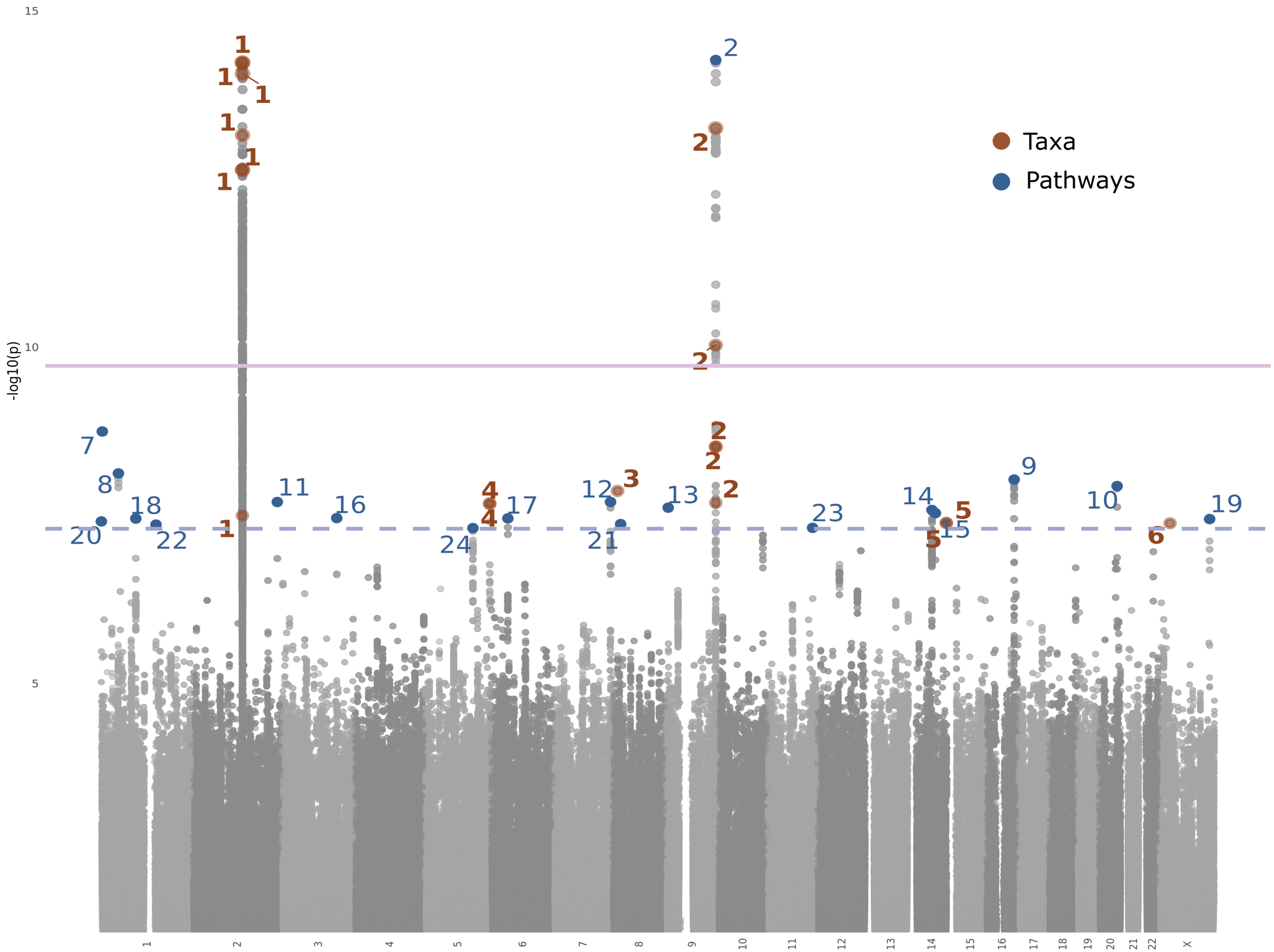
Relative abundances of microbiome features found to be associated with SNPs at the ABO locus are compared between the inferred blood types (O, A, B and AB) with O as reference group (panels **a**, **b** and **c**) and between secretors (light blue) and non-secretors (dark blue) stratified again by the blood types grouped by the presence (A/B/AB) or absence (O) of terminal sugars (panels **d**, **e** and **f**). The natural log–transformed relative abundance, adjusted by age and sex, and is shown using a violin plot in which the inner box represents the first and third quartiles of the distribution and the middle line represents the median. In the top panels, the distribution across all blood types is also significantly different for all features (ANOVA: $p = 6.7\text{x}10^{-14}$ for species *B. Bifidum*, $p = 5.4\text{x}10^{-9}$ for *LACTOSECAT-PWY* and $p = 1.4\text{x}10^{-13}$ for genus *Collinsella*). *P*-values are obtained from a two-sided Wilcoxon ranked test. S.: secretors, N.s.: non-secretors, ns: $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

Figure 4. Weighted Spearman correlation between estimated heritability and number of suggestive loci.

Each point represents one taxon or pathway. Bold red line represents the locally estimated scatterplot smoothing fit, with orange shaded bands indicating the 95% regression confidence intervals. The number of independent suggestive mbQTLs was obtained using LD pruning (**Methods**). **a)** Weighted Spearman correlation of number of suggestive mbQTLs and family-based heritability for microbiome taxa. **b)** Weighted Spearman correlation of number of suggestive mbQTLs and family-based heritability for microbiome functional pathways.

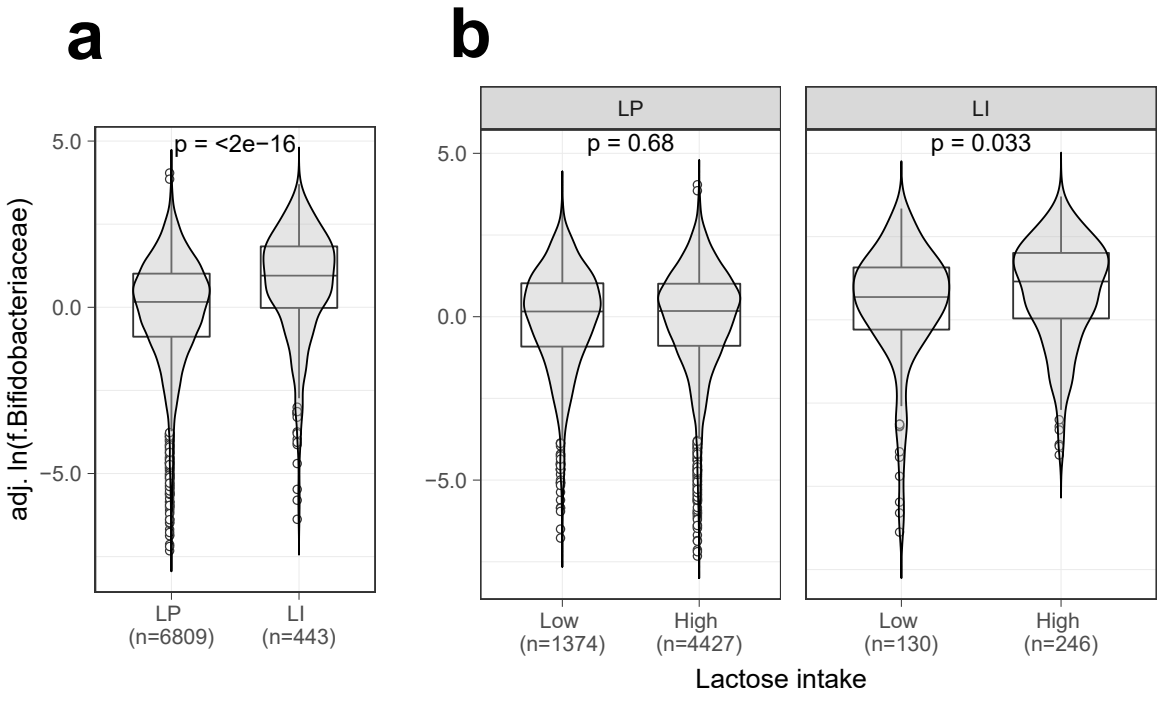
Figure 5. Power analysis taking into account bacterial presence levels

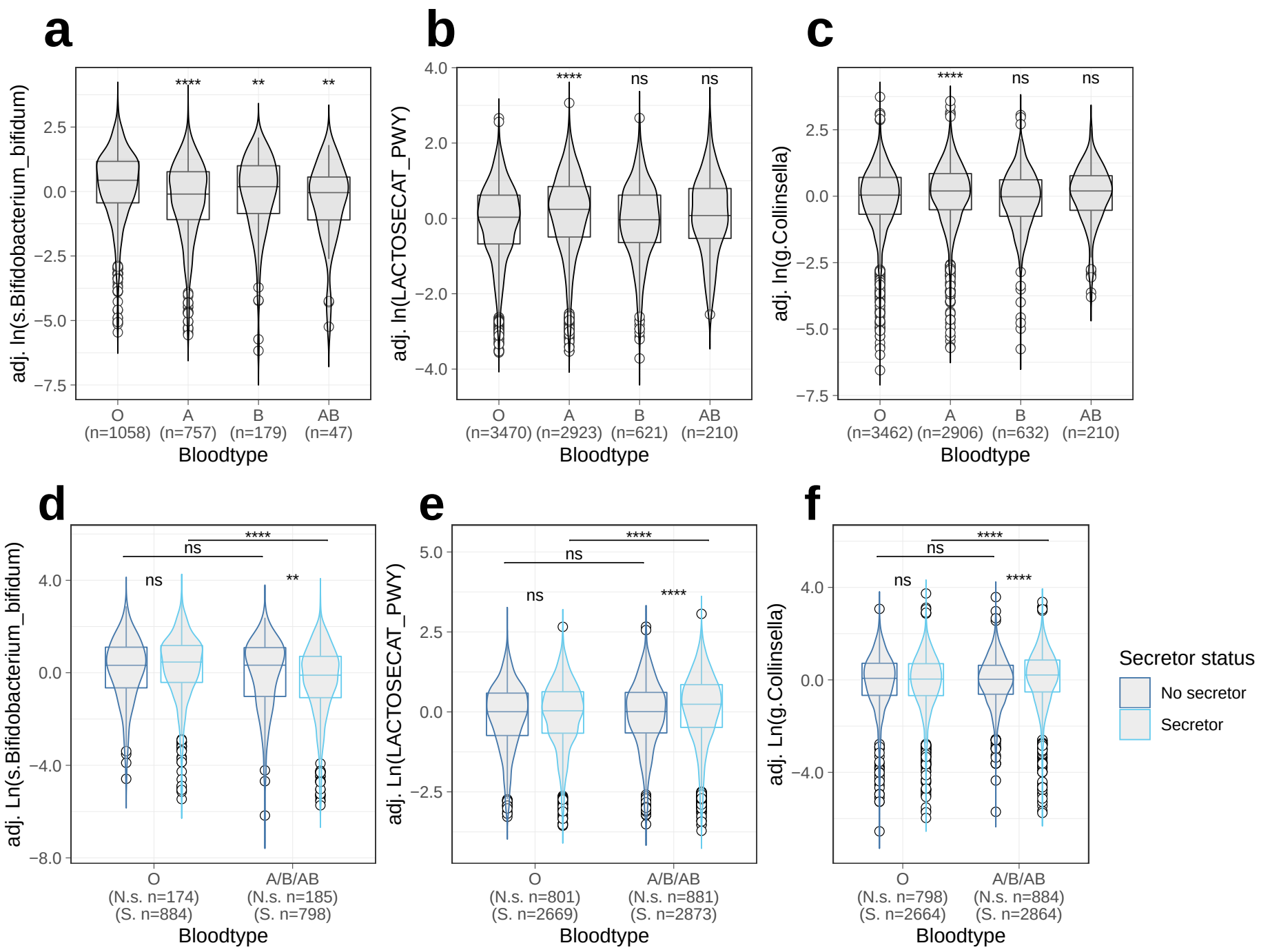
a) Statistical power (at alpha = $1x10^{-10}$) in our dataset for SNPs at the *ABO* and *LCT* loci, considering the associated taxonomies. 80% detection power is shown by the red horizontal line. **b**) Power (y-axis) to detect an effect at significance level alpha = $1x10^{-10}$ depending on the variance explained by a genetic variant (x-axis). Colored lines distinguish power under different levels of bacterial or pathway prevalence, which reduces effective sample size. **c**) Sample size needed to identify an association at 80% power level across different bacterial presence levels and alpha = $1x10^{-10}$. Horizontal dashed line indicates the sample size of the DMP cohort.

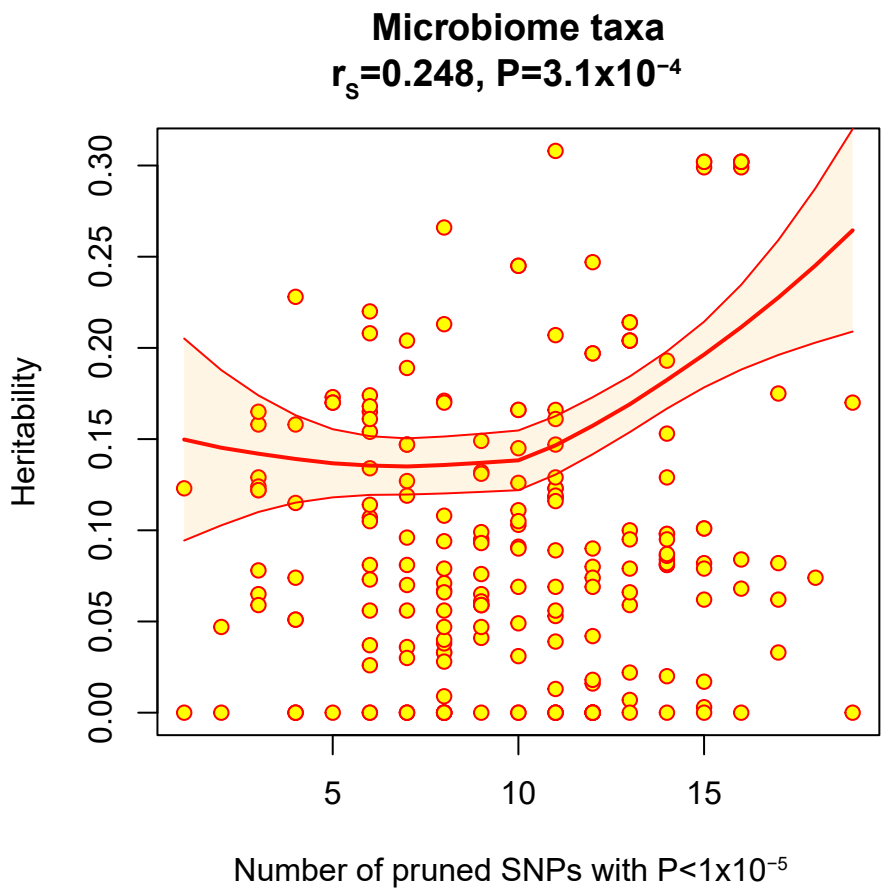


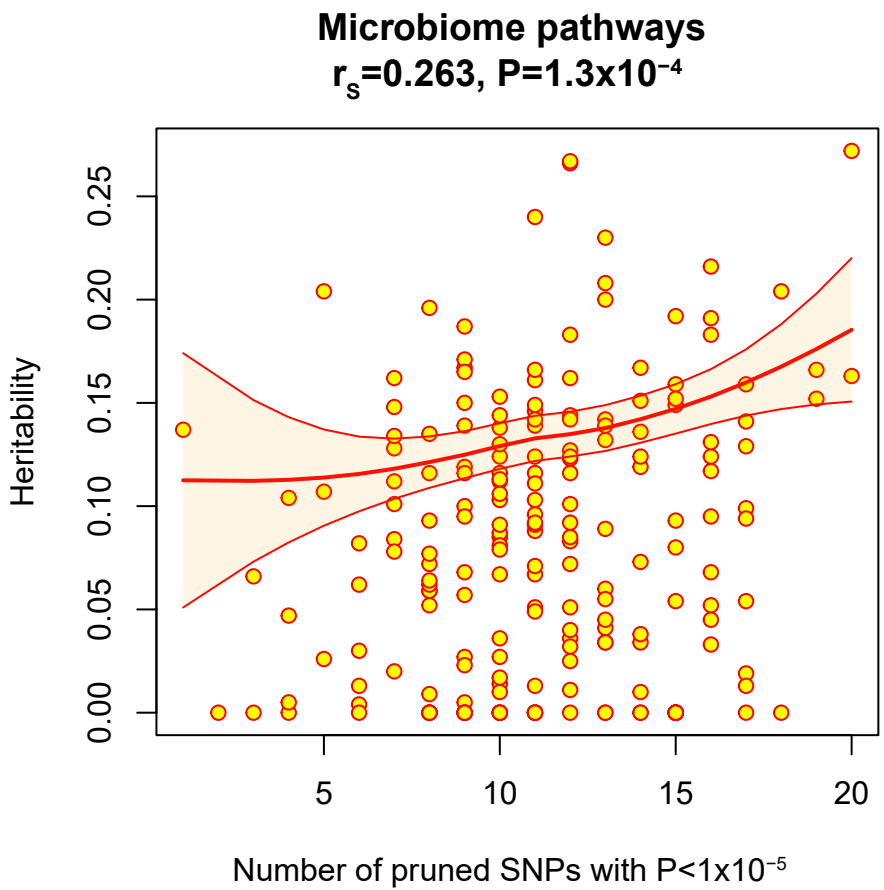
Locus ID	Genes (Variant function)	id	chr	pos	pval	Trait
1	*MCM6 (intronic);*	rs182549	2	136616754	5.86E-15	family Bifidobacteriaceae
1	*MCM6 (intronic);*	rs182549	2	136616754	5.91E-15	order Bifidobacteriales
1	*MCM6 (intronic);*	rs182549	2	136616754	8.59E-15	genus Bifidobacterium
2	*ABO (intronic)*	rs8176645	9	136149098	5.54E-14	species Bifidobacterium bifidum
1	*DARS1 (intronic)*	rs6754311	2	136707982	7.06E-14	species Bifidobacterium adolescentis
1	*MCM6 (intronic);*	rs182549	2	136616754	2.32E-13	class Actinobacteria
1	*MCM6 (intronic);*	rs182549	2	136616754	2.32E-13	phylum Actinobacteria
2	*ABO (intronic)*	rs550057	9	136146597	9.34E-11	genus Collinsella
2	*ABO (intronic)*	rs550057	9	136146597	3.01E-09	family Coriobacteriaceae
2	*ABO (intronic)*	rs550057	9	136146597	3.03E-09	order Coriobacteriales
2	*ABO (intronic)*	rs550057	9	136146597	2.05E-08	species Collinsella aerofaciens
3	*SGCZ (intron)*	rs28450200	8	15070111	1.37E-08	sècoes Alistipes sp. AP11
4	*COL23A1 (intron)*	rs73344869	5	177746345	2.10E-08	family Rikenellaceae
4	*COL23A1 (intron)*	rs73344869	5	177746345	2.14E-08	genus Alistipes
1	*RH3DM1 (missense)*	rs1446585	2	136407479	3.18E-08	species Bifidobacterium longum
5	*TNFAIP2 (intronic); LINC0067 (intronic)*	rs2282037	14	103588515	4.10E-08	class Negativicutes
5	*TNFAIP2 (intronic); LINC0067 (intronic)*	rs2282037	14	103588515	4.10E-08	order Selenomonadales
6	DMD (intron)	rs921896	X	32213962	4.15E-08	species Escherichia unclassified

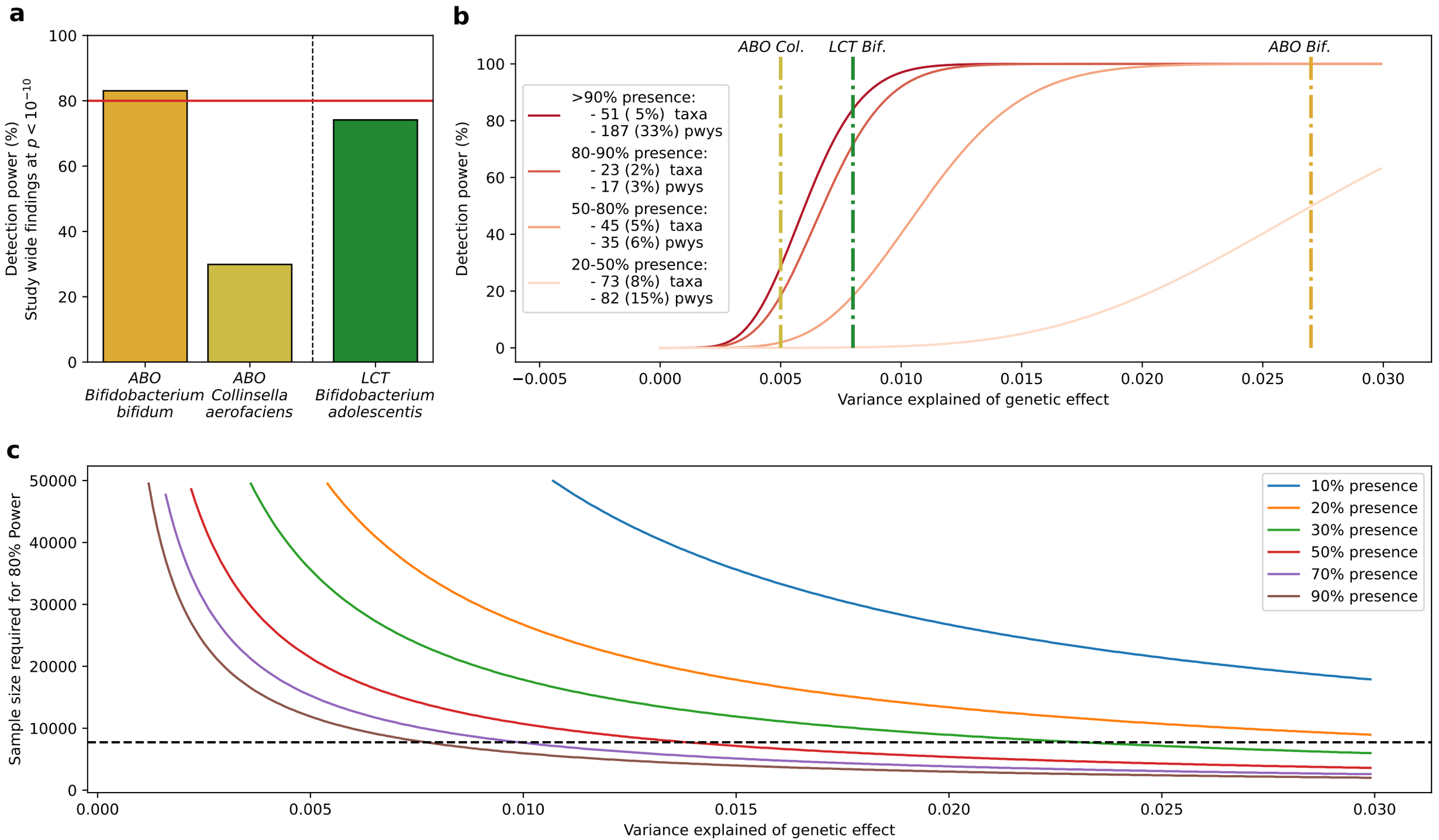
Locus ID	Genes (Variant function)	id	chr	pos	pval	Trait
2	*ABO (intronic)*	rs507666	9	136149399	5.38E-15	LACTOSECAT-PWY: lactose and galactose degradation I
7	intergenic	rs7513344	1	4220095	1.79E-09	PWY-6151: S-adenosyl L methionine cycle I
8	*AC105942.1 (intronic)*	rs10159356	1	48103139	7.52E-09	PWY-REDCITCYC: TCA cycle VIII (helicobacter)
9	*WWOX (intron)*	rs9927590	16	78318743	9.28E-09	PWY-5088: L-glutamate degradation VIII to propanoate
10	*SALL4 (intron)*	rs79676117	20	50415961	1.16E-08	PWY-7234: inosine-5'-phosphate biosynthesis III
11	*AC010149.1 (intron)*	rs11898768	2	231419306	2.00E-08	PWY-6708: ubiquinol-8 biosynthesis
12	closest genes: INSIG and BLACE	rs36182340	7	155118470	2.00E-08	P105-PWY: TCA cycle IV (2-oxoglutarate decarboxylase)
13	*LINC02851 (upstream)*	rs820496	9	6703997	2.43E-08	ASPASN-PWY: superpathway of L-aspartate and L-asparagine biosynthesis
14	*SYNE2 (intronic)*	rs17824268	14	64467727	2.63E-08	PWY-6612: superpathway of tetrahydrofolate biosynthesis
15	*NUMB (intronic)*	rs148280750	14	73763641	2.93E-08	PYRIDNUCSYN-PWY: NAD biosynthesis I from aspartate
16	closest gene: TSC22D2	rs1584586	3	150194351	3.47E-08	PWY-841: superpathway of purine nucleotides de-novo biosynthesis I
17	RCAN2 (intron)	rs59657730	6	46422725	3.50E-08	GLYCOCAT.PWY: glycogen degradation I (bacterial)
18	*closest genes: CNN3 and ALG14*	rs12137024	1	95409858	3.53E-08	HISDEG-PWY: L-histidine degradation I
19	*AL451048.1 (intron)*	rs11795855	X	140027569	3.59E-08	ENTBACSYN-PWY: enterobactin biosynthesis
20	CDK11B	rs11486024	1	1655474	3.89E-08	TRPSYN PWY: L-tryptophan biosynthesis
21	SLC25A37 (intron)	rs2137304	8	23415240	4.26E-08	PWY-6353: purine nucleotides degradation II (aerobic)
22	closest gene: RPRD2	rs78778914	1	150449701	4.34E-08	HISTSYN PWY: L-histidine biosynthesis
23	GRAMD1B	rs2714053	11	123312063	4.85E-08	PWY-6147: 6-hydroxymethyl dihydropterin diphosphate biosynthesis I
24	closest genes: IL4 and KIF3A (eQTL)	rs11242123	5	132025487	4.89E-08	PWY-7209: superpathway of pyrimidine ribonucleosides degradation


a
b
p = <2e−16
5.0
0.0
−5.0
adj. ln(f.Bifidobacteriaceae)
LP
(n=6809)
LI
(n=443)
LP
LI
p = 0.68
p = 0.033
Low
(n=1374)
High
(n=4427)
Low
(n=130)
High
(n=246)
Lactose intake


a
adj. ln(s.Bifidobacterium_bifidum)
****
**
**
O (n=1058)
A (n=757)
B (n=179)
AB (n=47)
Bloodtype
b
adj. ln(LACTOSECAT_PWY)
****
ns
ns
O (n=3470)
A (n=2923)
B (n=621)
AB (n=210)
Bloodtype
c
adj. ln(g.Collinsella)
****
ns
ns
O (n=3462)
A (n=2906)
B (n=632)
AB (n=210)
Bloodtype
d
adj. Ln(s.Bifidobacterium_bifidum)
ns
****
ns
**
O (N.s. n=174) (S. n=884)
A/B/AB (N.s. n=185) (S. n=798)
Bloodtype
e
adj. Ln(LACTOSECAT_PWY)
ns
****
ns
****
O (N.s. n=801) (S. n=2669)
A/B/AB (N.s. n=881) (S. n=2873)
Bloodtype
f
adj. Ln(g.Collinsella)
ns
****
ns
****
O (N.s. n=798) (S. n=2664)
A/B/AB (N.s. n=884) (S. n=2864)
Bloodtype
Secretor status
No secretor
Secretor


Microbiome taxa
r_s=0.248, P=3.1x10^{-4}
Heritability
0.00
0.05
0.10
0.15
0.20
0.25
0.30
5
10
15
Number of pruned SNPs with P<1x10^{-5}


Microbiome pathways
r_s=0.263, P=1.3x10^{-4}
Heritability
0.00
0.05
0.10
0.15
0.20
0.25
5
10
15
20
Number of pruned SNPs with P<1x10^{-5}


a
Detection power (%)
Study wide findings at $p < 10^{-10}$
100
80
60
40
20
0
ABO
Bifidobacterium
bifidum
ABO
Collinsella
aerofaciens
LCT
Bifidobacterium
adolescentis
b
ABO Col.
LCT Bif.
ABO Bif.
>90% presence:
- 51 (5%) taxa
- 187 (33%) pwys
80-90% presence:
- 23 (2%) taxa
- 17 (3%) pwys
50-80% presence:
- 45 (5%) taxa
- 35 (6%) pwys
20-50% presence:
- 73 (8%) taxa
- 82 (15%) pwys
Detection power (%)
−0.005
0.000
0.005
0.010
0.015
0.020
0.025
0.030
Variance explained of genetic effect
c
Sample size required for 80% Power
50000
40000
30000
20000
10000
0
10% presence
20% presence
30% presence
50% presence
70% presence
90% presence
0.000
0.005
0.010
0.015
0.020
0.025
0.030
Variance explained of genetic effect