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Large-scale association analyses identify host factors influencing human gut microbiome composition

To study the effect of host genetics on gut microbiome composition, the MiBioGen consortium curated and analyzed genome-wide genotypes and 16S fecal microbiome data from 18,340 individuals (24 cohorts). Microbial composition showed high variability across cohorts: only 9 of 410 genera were detected in more than 95% of samples. A genome-wide association study of host genetic variation regarding microbial taxa identified 31 loci affecting the microbiome at a genome-wide significant ($P < 5 \times 10^{-8}$) threshold. One locus, the lactase (*LCT*) gene locus, reached study-wide significance (genome-wide association study signal: $P = 1.28 \times 10^{-20}$), and it showed an age-dependent association with *Bifidobacterium* abundance. Other associations were suggestive ($1.95 \times 10^{-10} < P < 5 \times 10^{-8}$) but enriched for taxa showing high heritability and for genes expressed in the intestine and brain. A phenome-wide association study and Mendelian randomization identified enrichment of microbiome trait loci in the metabolic, nutrition and environment domains and suggested the microbiome might have causal effects in ulcerative colitis and rheumatoid arthritis.

The gut microbiome is an integral part of the human holobiont. In recent years, many studies have highlighted the link between its perturbations and immune, metabolic, neurologic and psychiatric traits, drug metabolism and cancer[1]. Environmental factors, like diet and medication, play a substantial role in shaping the gut microbiome composition[2–4], although twin, family and population-based studies have shown that the genetic component also plays a role in determining gut microbiota composition, and a proportion of bacterial taxa are heritable[5,6].

Several studies[7–9] have investigated the effect of genetics on microbiome composition through genome-wide association studies (GWAS) and identified dozens of associated loci. However, little replication across these studies has been observed so far[10,11]. This may be due to a number of factors. First, methodological differences in the collection, processing and annotation of stool microbiota are known to have strong effects on the microbiome profiles obtained[12–14] and can generate heterogeneity and a lack of reproducibility across studies. Second, most association signals are rather weak, which suggests that existing studies of 1,000–2,000 samples[7–9] are underpowered. Finally, some of the GWAS signals related to microbiome compositions may be population specific, that is, they may represent bona fide population differences in genetic structure and/or environment.

To address these challenges and obtain valuable insights into the relationship between host genetics and microbiota composition, we set up the international consortium MiBioGen[11]. In this study, we coordinated 16S ribosomal RNA (rRNA) gene sequencing profiles and genotyping data from 18,340 participants from 24 cohorts from the United States, Canada, Israel, South Korea, Germany, Denmark, the Netherlands, Belgium, Sweden, Finland and the United Kingdom. We performed a large-scale, multiancestry, genome-wide meta-analysis of the associations between autosomal human genetic variants and the gut microbiome. We explored the variation of microbiome composition across different populations and investigated the effects of differences in methodology on the microbiome data. Through the implementation of a standardized pipeline, we then performed microbiome trait loci (mbTL) mapping to identify genetic loci that affect the relative abundance (microbiome quantitative trait loci, or mbQTLs) or presence (microbiome binary trait loci, or mbBTLs) of microbial taxa. Finally, we focused on the biological interpretation of GWAS findings through gene-set enrichment analysis (GSEA), phenome-wide association studies (PheWAS) and Mendelian randomization (MR) approaches.

Results

Landscape of microbiome composition across cohorts. Our study included cohorts that were heterogeneous in terms of ancestry, age, male/female ratio and microbiome analysis methodology. Twenty cohorts included samples of single ancestry, namely European (16 cohorts; $n = 13{,}266$), Middle Eastern (1 cohort; $n = 481$), East Asian (1 cohort; $n = 811$), American Hispanic/Latin (1 cohort; $n = 1{,}097$) and African American (1 cohort; $n = 114$), whereas four cohorts included samples from multiple ancestries ($n = 2{,}571$; Supplementary Note and Supplementary Tables 1 and 2).

Twenty-two cohorts comprised adult or adolescent individuals ($n = 16{,}632$), and two cohorts consisted of children ($n = 1{,}708$). The microbial composition was profiled by targeting three distinct variable regions of the 16S rRNA gene: V4 (10,413 samples; 13 cohorts), V3–V4 (4,211 samples; 6 cohorts) and V1–V2 (3,716 samples, 5 cohorts; Fig. 1a). To account for differences in sequencing depth, all datasets were rarefied to 10,000 reads per sample. Next, we performed taxonomic classification using direct taxonomic binning instead of operational taxonomic unit (OTU) clustering methods (Methods)[11,15,16].

In general, cohorts varied in their microbiome structure at multiple taxonomic levels (Fig. 1b–g). This variation may largely be driven by the heterogeneity between populations and differences in technical protocols (Supplementary Tables 1–3). Combining all samples ($n = 18{,}340$) resulted in a total richness of 385 genus-level taxonomic groups that had a relative abundance higher than 0.1% in at least one cohort. This observed total richness appeared to be below the estimated saturation level (Fig. 1b), suggesting that a further increase in sample size and a higher sequencing depth are needed to capture the total gut microbial diversity (Fig. 1d).

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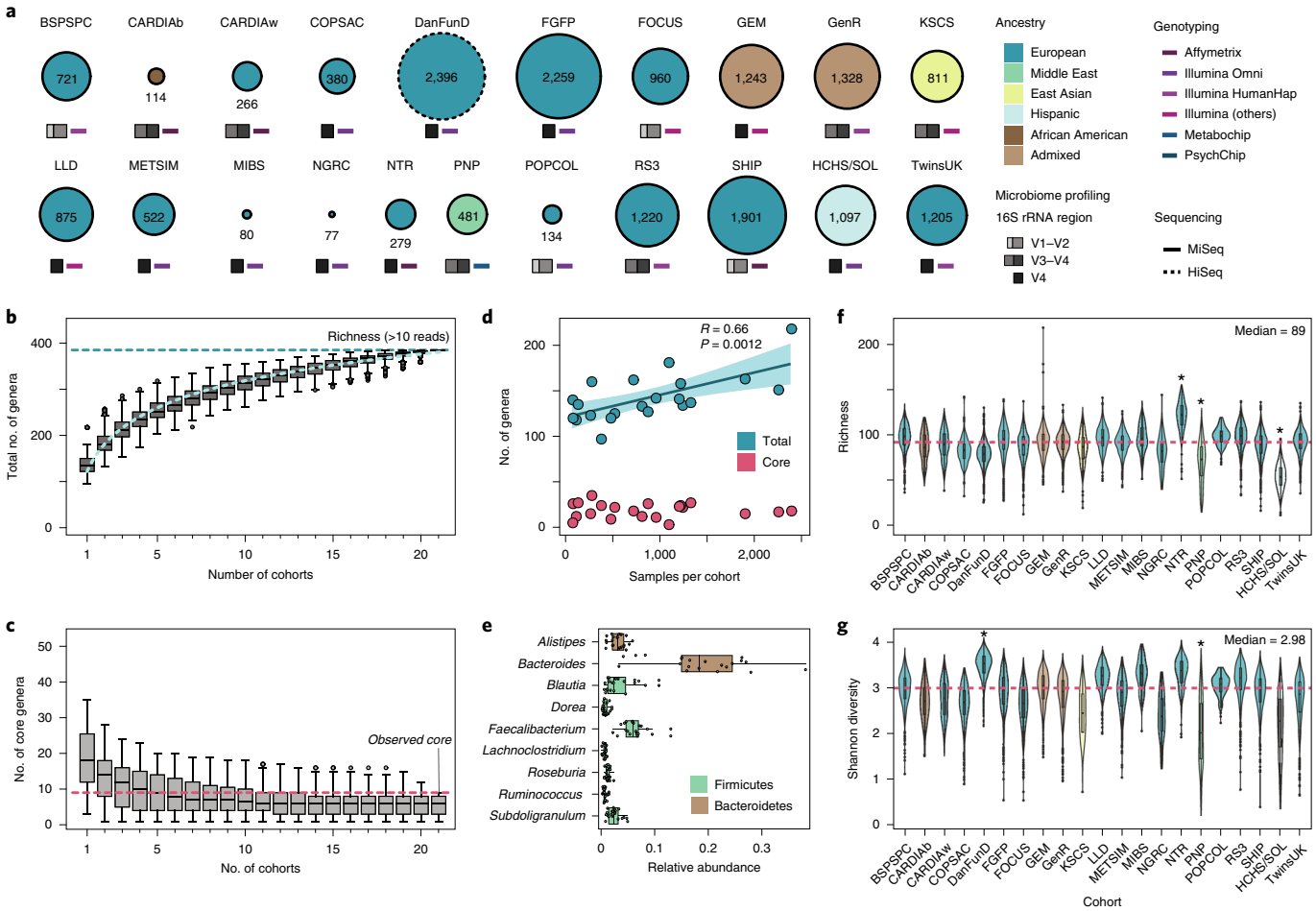


Fig. 1 | Diversity of microbiome composition across the MiBioGen cohorts. a, Sample size, ancestry, genotyping array and 16S rRNA gene profiling method. The SHIP/SHIP-TREND and GEM_v12/GEM_v24/GEM_ICHIP subcohorts were combined in SHIP and GEM, respectively (Methods; see Supplementary Note for cohort abbreviations), resulting in a total of 21 cohorts. **b**, Total richness (number of genera with mean abundance over 0.1%, that is, 10 reads of 10,000 rarefied reads) by number of cohorts investigated. **c**, Number of core genera (genera present in >95% of samples from each cohort) by number of cohorts investigated. **d**, Pearson correlation of cohort sample size with total number of genera. Confidence bands represents the standard error (s.e.) of the regression line. **e**, Unweighted mean relative abundance of core genera across the entire MiBioGen dataset. **f**, Per-sample richness across the 21 cohorts. **g**, Diversity (Shannon index) across the 21 cohorts, with the DanFund and PNP cohorts presenting higher and lower diversity in relation to the other cohorts. In **f** and **g**, asterisks indicate cohorts that differed significantly from all the others (pairwise Wilcoxon rank-sum test; false discovery rate < 0.05). For all box plots (**b**, **c** and **e**), the central line, box and whiskers represent the median, interquartile range (IQR) and 1.5 times the IQR, respectively.

As expected, the core microbiota (the number of bacterial taxa present in over 95% of individuals) decreased with the inclusion of additional cohorts (Fig. 1c and Methods). The core microbiota comprises nine genera, of which seven were previously identified as such[3], and the genera *Ruminococcus* and *Lachnoclostridium* (Fig. 1e). Of these nine genera, the most abundant genus was *Bacteroides* (18.65% (standard deviation (SD): 8.65%)), followed by *Faecalibacterium* (6.19% (SD: 2.35%)), *Blautia* (3.36% (SD: 2.84%)) and *Alistipes* (3.05% (SD: 1.47%)). Among the European cohorts that compose the largest genetically and environmentally homogeneous cluster, the core microbiota also included *Ruminiclostridium*, *Fusicatenibacter*, *Butyricicoccus* and *Eubacterium*, genera that typically produce short-chain fatty acids[17].

The DNA extraction method was the principal contributor to heterogeneity, with a nonredundant effect size of 29% on the microbiome variation (measured as average genus abundance per cohort; stepwise distance-based redundancy analysis adjusted R squared ($R^2\text{adj}_{\text{DNAext}}$) = 0.27, adjusted P value (P_{adj}) = 7×10^{-4}; Supplementary Table 4). Richness and Shannon diversity also differed significantly across cohorts. The cohorts with the lowest richness (HCHS/SOL) and highest diversity (DanFund) used specific DNA extraction kits that were not used by other studies, possibly contributing to their outlying alpha diversities (Fig. 1f,g and Supplementary Table 3). Overall, the 16S rRNA domain sequence and the DNA extraction methods used, together with cohort ancestry, accounted for 32.74% of richness variance.

Given the high heterogeneity of microbial composition across cohorts, we applied both per-cohort and whole-study filters for taxa inclusion in GWAS (Methods).

Heritability of microbial taxa and alpha diversity. We performed estimation of heritability (H^2) of gut microbiome composition based on the two twin cohorts included in our study (Supplementary Table 5). The TwinsUK cohort, composed of 1,176 samples, including 169 monozygotic (MZ) and 419 dizygotic (DZ) twin pairs, was used to estimate H^2 using the ACE (additive genetic variance (A)/shared environmental factors (C)/non-shared factors plus error (E)) model. The Netherlands Twin Registry (NTR) cohort (only MZ twins; n = 312, 156

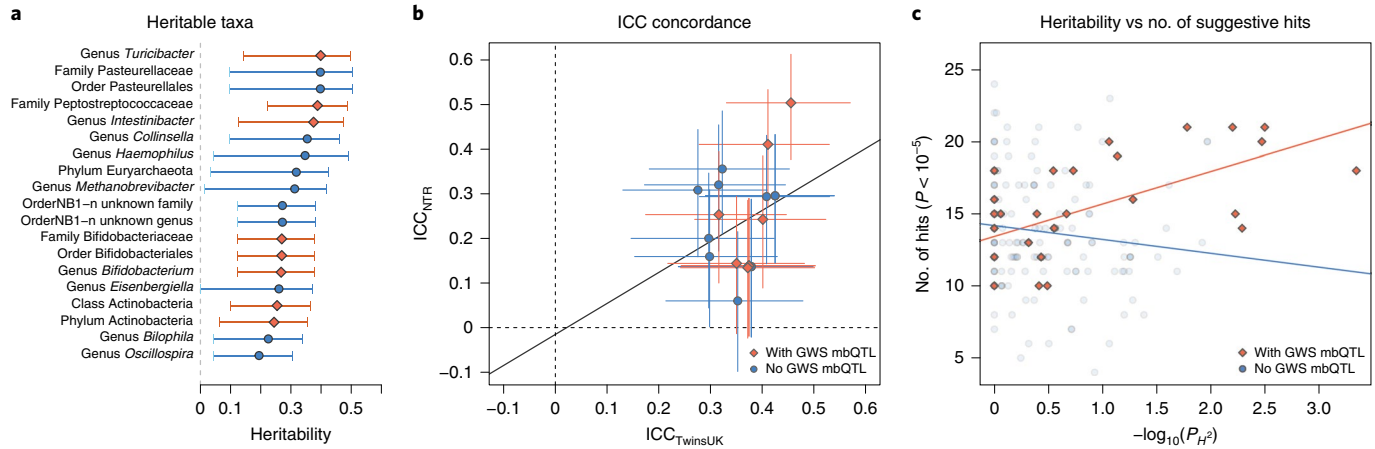


Fig. 2 | Heritability of microbiome taxa and its concordance with mbQTL mapping. a, Microbial taxa that showed significant heritability in the TwinsUK cohort (ACE model; nominal $P<0.05$, no adjustment for multiple comparison). Taxa with at least one genome-wide significant (GWS) mbQTL hit are marked in red. Only taxa present in more than 10% of pairs (>17 MZ pairs and >41 DZ pairs) are shown. Circles and diamonds represent heritability values. Error bars represent 95% CIs. **b**, Correlation of MZ ICC between TwinsUK and NTR cohort. Only taxa with significant heritability (ACE model $P<0.05$) that were present in both TwinsUK and NTR are shown. Red and blue dots indicate bacterial taxa with and without GWS mbQTLs ($P<5\times10^{-8}$), respectively. Segments represent 95% CIs. **c**, Correlation between heritability significance ($-\log_{10} P_{H2}$ TwinsUK) and the number of loci associated with microbial taxon at relaxed threshold ($P_{mbQTL}<1\times10^{-5}$). Taxa with at least one GWS-associated locus are marked in red. Error bars represent 95% CIs.

pairs) was used to replicate the MZ intraclass correlation coefficient (ICC). None of the alpha diversity metrics (Shannon, Simpson and inverse Simpson) showed evidence for heritability ($A<0.01$, $P=1$). Among the 159 bacterial taxa that were present in more than 10% of twin pairs, 19 taxa showed evidence for heritability ($P_{nominal}<0.05$; Fig. 2a). The ICC showed concordance between TwinsUK and NTR for these 19 bacterial taxa ($R=0.25$, $P=0.0018$; Fig. 2b).

The SNP-based heritability calculated from mbQTL summary statistics using linkage disequilibrium (LD) score regression showed two bacterial taxa, genus *Ruminiclostridium* 9 and family Peptostreptococcaeae, passing the significance threshold given the number of 211 taxa tested ($Z<3.68$; Supplementary Table 5). The results of the SNP-based heritability and twin-based heritability showed significant correlation across the tested taxa ($R=0.244$, $P=7.2\times10^{-4}$).

Thirty-one loci associated with gut microorganisms through GWAS. First, we studied the genetic background of the alpha diversity (Simpson, inverse Simpson and Shannon diversity indices). We identified no significant hits in the meta-analysis of GWAS ($P>5\times10^{-8}$; Supplementary Table 6 and Supplementary Fig. 1), in line with the observed lack of heritability for these indices.

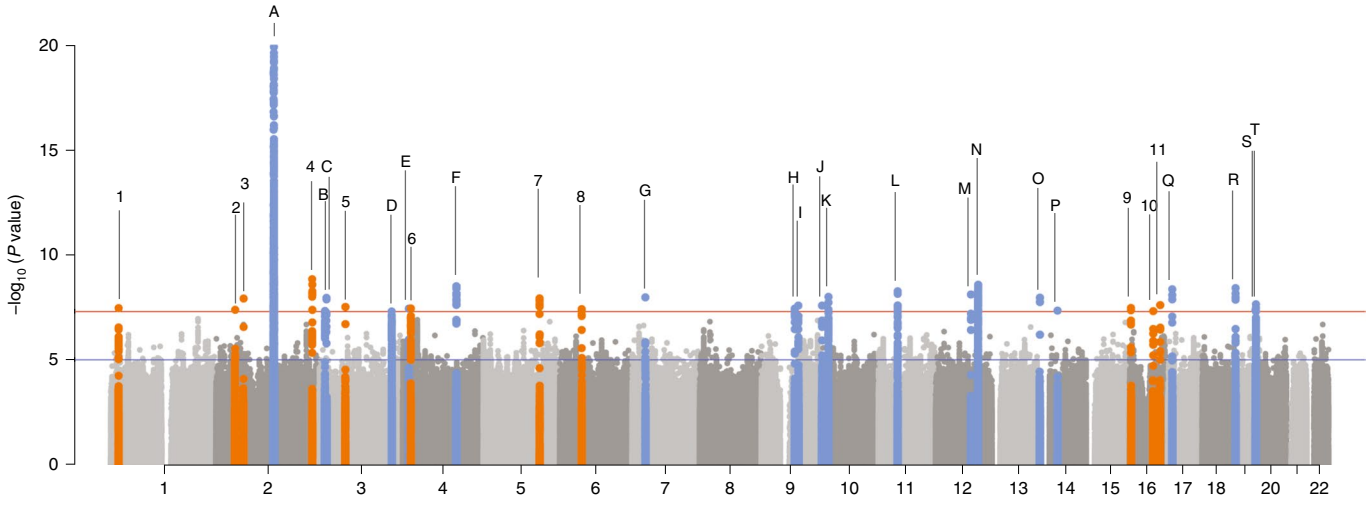
Next, we used two separate GWAS meta-analysis approaches[18–20] to explore the effect of host genetics on the abundance levels (mbQTL) or presence/absence (mbBTL) of bacterial taxa in the gut microbiota (Methods).

In total, 18,340 samples and 211 taxa were included in the mbQTL mapping analysis (Methods and Supplementary Table 3). We identified genetic variants that mapped to 20 distinct genetic loci associated with the abundance of 27 taxa (Fig. 3, Supplementary Figs. 2 and 3 and Supplementary Tables 7 and 8). MbBTL mapping covered 177 taxa, and 10 loci were found to be associated with presence/absence of bacterial taxa (Fig. 3 and Supplementary Tables 7 and 9). For one taxon, family Peptococcaceae, two independent mbBTLs were detected (Fig. 3 and Supplementary Table 7). Two of 31 mbTLs showed heterogeneity in mbTL effect sizes (Supplementary Note).

In both the mbQTL and mbBTL mapping, only 1 of 31 loci (*LCT* locus: *Bifidobacterium*; $P=8.63\times10^{-21}$) passed the strict correction for the number of taxa tested ($P<1.95\times10^{-10}$ for 257 taxa included in the analysis). However, the remaining loci included functionally relevant variants (that is, the *FUT2* gene suggested by earlier studies[21]) and, overall, showed concordance with the heritability of microbial taxa. Seven of the nine taxa that showed the strongest evidence for heritability in the TwinsUK cohort ($P<0.01$) also have genome-wide significant mbTLs (Fig. 2b). For the taxa with genome-wide significant mbTLs, the number of independent loci associated with a relaxed threshold of 1×10^{-5} strongly correlated with heritability significance ($R=0.62$, $P=1.9\times10^{-4}$; Fig. 2c), suggesting that more mbTLs would be identified for this group of bacteria using a larger sample size.

***LCT* mbQTL effect shows age and ancestry heterogeneity.** The strongest association signal was seen for variants located in a large block of about 1.5 Mb at 2q21.3, which includes the *LCT* gene and 12 other protein-coding genes. This locus has previously been associated with the abundance of *Bifidobacterium* in Dutch[7], UK[6] and US[22] cohorts. Previous studies have also shown a positive correlation of *Bifidobacterium* abundance with the intake of milk products, but only in individuals homozygous for the low-function LCT haplotype, thereby indicating that gene–diet interaction regulates *Bifidobacterium* abundance[7]. In our study, the strongest association was seen for rs182549 ($P=1.28\times10^{-20}$), which is a perfect proxy for the functional *LCT* variant rs4988235 ($r^2=0.996$; $D'=1$ in European populations). This association showed evidence for heterogeneity across cohorts ($I^2=62.73\%$, Cochran's Q $P=1.4\times10^{-4}$). A leave-one-out strategy showed that the Copenhagen Prospective Studies on Asthma in Childhood ($COPSAC_{2010}$) cohort, which includes children with an age range of 4–6 years, contributed the most to the detected heterogeneity (Fig. 4a,b and Supplementary Table 2). When this study was excluded from the meta-analysis, the heterogeneity was reduced ($I^2=51.9\%$, Cochran's Q $P=0.004$). A meta-regression analysis showed that linear effects of age and ancestry accounted for 11.84% of this heterogeneity. Including quadratic and cubic terms of age in the model explained 39.22% of the heterogeneity, and the residual heterogeneity was low (Cochran's Q $P=0.01$; Fig. 4c).

Following these observations, we decided to investigate the effect of age and ancestry in the multiancestry GEM cohort, comprising 1,243 individuals with an age range between 6 and 35 years, of which nearly half of the participants are 16 years or younger. Our



Locus	P value	Type	Chr	rsID	Bacteria
A	1.28×10^{-20}	Quant	2	rs182549	Genus *Bifidobacterium*
B	4.66×10^{-8}	Quant	3	rs9864379	Order Gastranaerophilales
C	1.10×10^{-8}	Quant	3	rs75754569	Genus *Peptococcus*
D	4.88×10^{-8}	Quant	3	rs4428215	Family Oxalobacteraceae
E	3.55×10^{-8}	Quant	4	rs10805326	Genus *Intestinibacter*
F	3.06×10^{-9}	Quant	4	rs11098863	Genus *Enterorhabdus*
G	1.04×10^{-8}	Quant	7	rs17159861	Genus group *Eubacterium coprostanoligenes*
H	3.57×10^{-8}	Quant	9	rs602075	Genus *Allisonella*
I	2.57×10^{-8}	Quant	9	rs736744	Genus *Oxalobacter*
J	2.55×10^{-8}	Quant	10	rs12781711	Genus *Ruminococcaceae* UCG013
K	9.80×10^{-9}	Quant	10	rs61841503	Family Peptostreptococcaceae
L	5.29×10^{-9}	Quant	11	rs10769159	Genus *Ruminococcus1*
M	7.57×10^{-9}	Quant	12	rs12320842	Genus *Faecalibacterium*
N	2.58×10^{-9}	Quant	12	rs11110281	Genus *Streptococcus*
O	1.08×10^{-8}	Quant	13	rs7322849	Genus *Bifidobacterium*
P	4.42×10^{-8}	Quant	14	rs8009993	Genus *Ruminococcaceae* UCG009
Q	4.31×10^{-9}	Quant	17	rs7221249	Genus *Erysipelatoclostridium*
R	3.74×10^{-9}	Quant	19	rs67476743	Genus *Tyzzerella3*
S	4.26×10^{-8}	Quant	19	rs830151	Genus *Candidatus Soleaferrea*
T	2.21×10^{-8}	Quant	19	rs35866622	Genus group *Ruminococcus torques*
1	3.34×10^{-8}	Binary	1	rs555115	Genus *Turicibacter*
2	4.07×10^{-8}	Binary	2	rs57440956	Genus *Ruminiclostridium6*
3	1.17×10^{-8}	Binary	2	rs56081657	Genus *Odoribacter*
4	1.42×10^{-9}	Binary	2	rs7574352	Family Peptococcaceae
5	2.98×10^{-8}	Binary	3	rs17066404	Family Peptococcaceae
6	3.53×10^{-8}	Binary	4	rs207281	Genus *Enterorhabdus*
7	1.16×10^{-8}	Binary	5	rs62373497	Genus *Coprococcus1*
8	3.79×10^{-8}	Binary	6	rs9474033	Family Pseudomonadaceae
9	3.34×10^{-8}	Binary	16	rs437867	Genus *Lachnospiraceae* UCG010
10	4.67×10^{-8}	Binary	16	rs17319026	Genus *Anaerostipes*
11	2.42×10^{-8}	Binary	16	rs11647069	Family Bacteroidales S24.7

Fig. 3 | Manhattan plot of the mbTL mapping meta-analysis results. MbQTLs are indicated by letters. MbBTLs are indicated by numbers. For mbQTLs, the Spearman correlation test (two-sided) was used to identify loci that affect the covariate-adjusted abundance of bacterial taxa, excluding samples with zero abundance. For mbQTLs, *P* values (two-sided) were calculated by logistic regression. Horizontal lines define nominal genome-wide significance ($P = 5 \times 10^{-8}$; red) and suggestive genome-wide ($P = 1 \times 10^{-5}$; blue) thresholds.

analysis showed a significant SNP–age interaction on the level of *Bifidobacterium* abundance ($P < 0.05$; Methods). Individuals homozygous for the NC_000002.11:g.136616754CC (rs182549) genotype showed a higher abundance of the genus *Bifidobacterium* in the adult group, but not in the younger group (Fig. 4d). The age–genotype interaction was significant in the GEM_v12 and GEM_ICHIP subcohorts, both comprising mostly individuals of European ancestry, while the GEM_v24 cohort is mainly composed of individuals of different Israeli subancestries (Methods) who live in Israel, showed neither an mbQTL effect (beta = −0.002 (95% confidence interval (CI): −0.21, 0.21)) nor an interaction with age ($P > 0.1$). The lack of an *LCT* mbQTL effect in adults was also observed in another Israeli cohort in the study (Personalized Nutrition Project (PNP): 481 adults, beta = −0.20 (95% CI: −0.61, 0.20)). Altogether, the cohorts that reported the lowest *LCT* effect sizes were the two cohorts of Israeli ancestry volunteered in Israel (GEM_v24 and PNP) and a child cohort (COPSAC: beta = −0.18 (95% CI: −0.36, −0.01)).

mbTLs are enriched for genes related to metabolism. Several loci detected at genome-wide significance level were enriched for genes related to metabolism.

In the mbQTL analysis, the *FUT2-FUT1* locus was associated with the abundance of the *Ruminococcus torques* genus group, a genus from the Lachnospiraceae family. The leading SNP (rs35866622 for *R. torques* group; $P = 2.21 \times 10^{-8}$) is a proxy for the functional variant rs601338 ($r^2 = 0.8$; $D' = 0.9$ in European populations) that introduces a stop codon in *FUT2* (ref. [23]). Another proxy of the functional *FUT2* SNP, rs281377, showed an association with the *Ruminococcus gnavus* genus group in the binary analysis; however, this signal was just above the genome-wide significance threshold ($P = 5.79 \times 10^{-8}$; Supplementary Table 9). *FUT2* encodes the enzyme alpha-1,2-fucosyltransferase, which is responsible for the secretion of fucosylated mucus glycans in the gastrointestinal mucosa[24]. Individuals homozygous for the stop codon (rs601338*A/A, non-secretors) do not express ABO antigens on the intestinal mucosa. We observed that the tagging NC_000019.9:g.49218060C > T (rs35866622 non-secretor) allele was associated with a reduced abundance of the *R. torques* group and a decreased presence of the *R. gnavus* group. *Ruminococcus sp.* are specialized in the degradation of complex carbohydrates[25], thereby supporting a link between genetic variation in the *FUT2* gene, levels of mucus glycans and the abundance of this taxa. When assessing the link between this variant and phenotypes in the LifeLines-DEEP (LLD; $n = 875$) and Flemish Gut Flora Project (FGFP; $n = 2{,}259$) cohorts (Methods), the strongest correlation for the *R. torques* group was seen with fruit intake (LLD: Spearman *R* (R_{Sp}) = −0.19, $P_{adj} = 3.1 \times 10^{-5}$; FGFP: $R_{Sp} = -0.10$, $P_{adj} = 1.4 \times 10^{-4}$; Supplementary Tables 10 and 11), in line with the association of *FUT2* with food preferences, as discussed in the results of the PheWAS (see below).

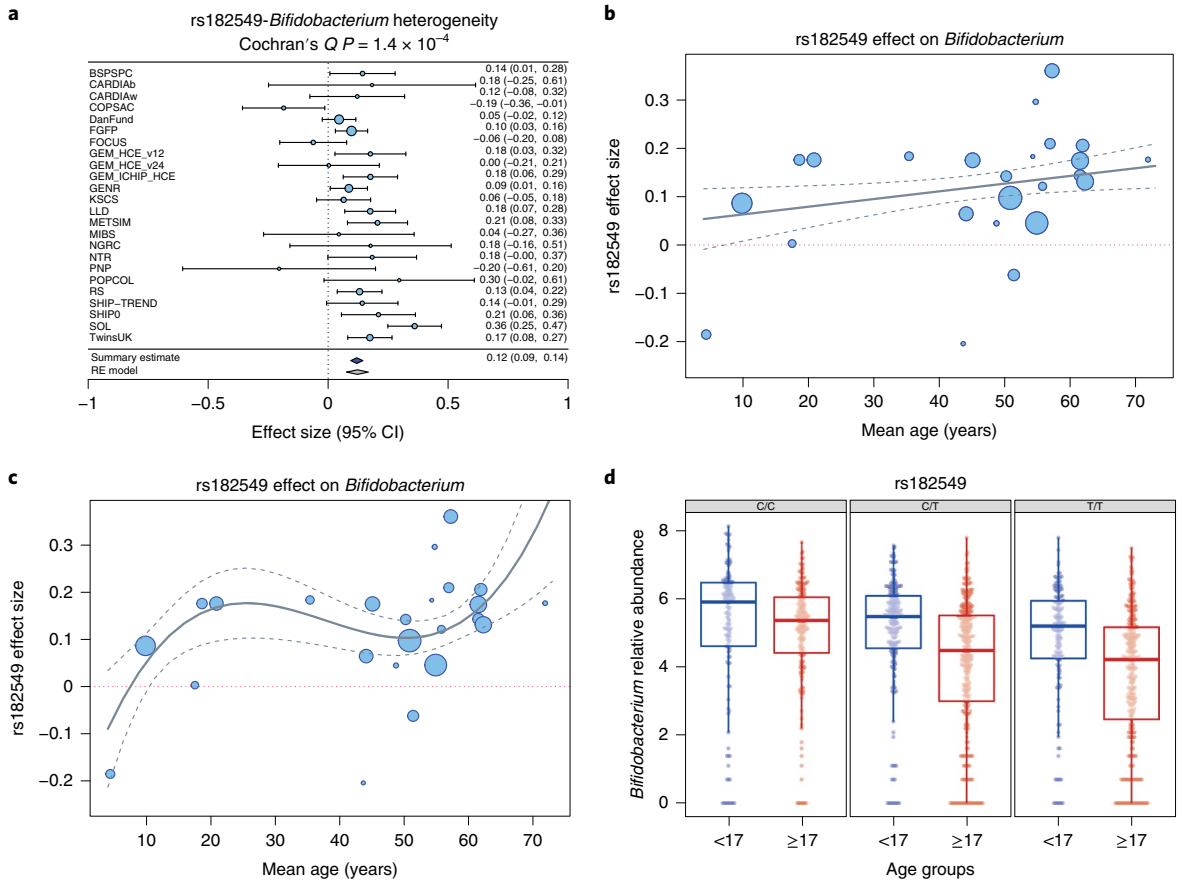


Fig. 4 | Association of the *LCT* locus (rs182549) with the genus *Bifidobacterium*. a, Forest plot of effect sizes of rs182549 and abundance of *Bifidobacterium*. Effect sizes and 95% CIs are defined as circles and error bars. Effect sizes were calculated from Spearman correlation *P* values (Methods). **b**, Meta-regression of the association of mean cohort age and mbQTL effect size. Confidence bands represent the s.e. of the meta-regression line. **c**, Meta-regression analysis of the effect of linear, squared and cubic terms of age on mbQTL effect size. Confidence bands represent the s.e. of the meta-regression line. **d**, Age dependence of mbQTL effect size in the GEM cohort. Blue boxes include samples in the age range of 6–16 years. Red boxes include samples with an age of ≥17 years. The C/C (rs182549) genotype is a proxy of the NC_000002.11:g.136608646=(rs4988235) allele, which is associated with functional recessive hypolactasia. The central line, box and whiskers represent the median, IQR and 1.5 times the IQR, respectively. Cohort abbreviations are available in the Supplementary Note.

Several other suggestive mbQTLs can be linked to genes potentially involved in host–microbiome cross-talk. One of them includes three SNPs in 9q21 (top SNP rs602075, $P=3.57\times10^{-8}$) associated with abundance of *Allisonella*. The 9q21 locus includes the genes *PCSK5*, *RFK* and *GCNT1*, of which *RFK* encodes the enzyme that catalyzes the phosphorylation of riboflavin (vitamin B_2) and *GCNT1* encodes a glycosyltransferase involved in biosynthesis of mucin. These products play major roles in the host–microbiota interactions within the intestine, where they are used by bacteria for their metabolism and involved in the regulation of the host immune defense[26]. Another association signal, 10p13 (rs61841503, $P=9.8\times10^{-9}$), which affects the abundance of the heritable family Peptostreptococcaceae, is located in the *CUBN* gene, the receptor for the complexes of cobalamin (vitamin B_{12}) with gastric intrinsic factor (the complex required for absorption of cobalamin). *CUBN* is expressed in the kidneys and the intestinal epithelium and is associated with B_{12}-deficient anemia and albuminuria[27]. Cobalamin is required for host–microbial interactions[28], and supplementation with cobalamin induced a substantial shift in the microbiota composition of an in vitro colon model[29]. These associations suggest that some members of the gut microbiome community might be affected by genetic variants that regulate the absorption and metabolism of vitamins B_2 and B_{12}.

Among mbBTLs, the strongest evidence for association was seen for a block of 10 SNPs (rs7574352, $P=1.42\times10^{-9}$) associated with the family Peptococcaceae, a taxon negatively associated with stool levels of the gut inflammation markers chromogranin A (LLD: $R_{Sp}=-0.31$, $P_{adj}=4.4\times10^{-18}$; Supplementary Table 10) and calprotectin (LLD: $R_{Sp}=-0.11$, $P_{adj}=0.058$) and with ulcerative colitis (FGFP: $R_{Sp}=-0.06$, $P_{adj}=0.09$; Supplementary Table 11). The association block is located in the intergenic region in the proximity (220 kb apart) of *IRF1*, which is involved in insulin resistance and susceptibility to type 2 diabetes[30].

Other highlights of identified mbTLs are available in the Supplementary Note.

GSEA, FUMA and PheWAS analysis. To explore the potential functions of the identified mbTLs, we performed functional mapping and annotation of genetic associations with the FUMA platform (Methods)[31], GSEA and PheWAS, followed by Bayesian colocalization analysis and genetic correlation of *Bifidobacterium* abundance to its PheWAS-related traits. FUMA of 20 mbQTLs returned 139 positional and eQTL genes. GSEA on these genes suggested an enrichment for genes expressed in the small intestine (terminal ileum) and brain (substantia nigra and putamen basal ganglia; Supplementary Fig. 4). The positional candidates for mbBTLs did not show any enrichment in GSEA analysis.

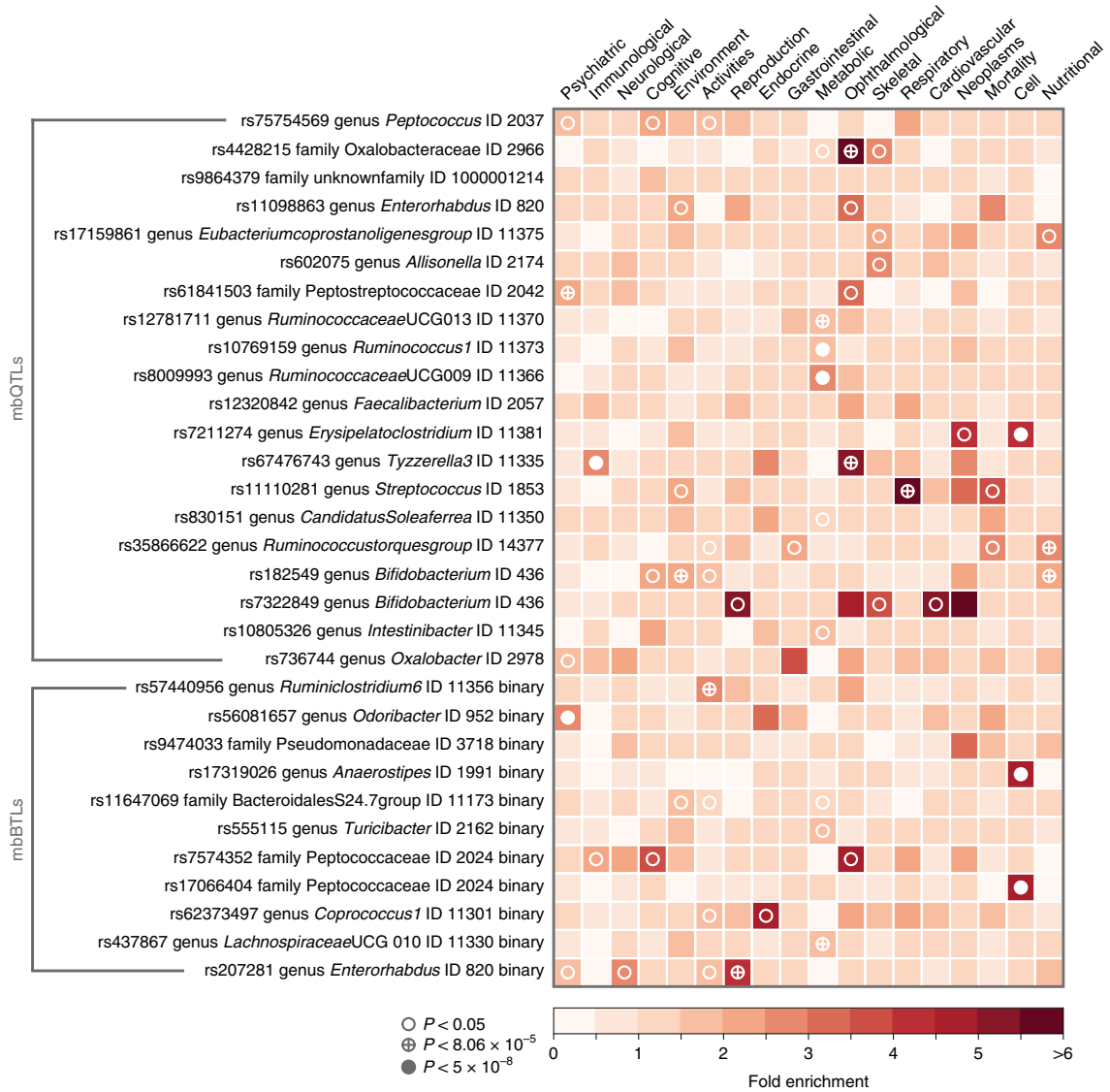


Fig. 5 | Phenome-wide association study domain enrichment analysis. The analysis covered top SNPs from 30 mbTLs and 20 phenotype domains. Three thresholds for multiple testing were used: 0.05, 8.06×10^{-5} (Bonferroni adjustment for number of phenotypes and genotypes studied) and 5×10^{-8} (an arbitrary genome-wide significance threshold). Only categories with at least one significant enrichment signal are shown.

To systematically assess the biological outcomes of the mbTLs, we examined the 31 mbTLs in the summary statistics for 4,155 complex traits and diseases using the GWASATLAS[32]. Five of 31 leading SNPs were associated with one or more phenotypes at $P<5\times10^{-8}$ (Supplementary Table 12): rs182549 (*LCT*) and rs35866622 (*FUT1/FUT2*), followed by rs4428215 (*FNDC3B*), rs11647069 (*PMFBP1*) and rs9474033 (*PKHD1*).

The variant showing the highest pleiotropy, rs182549 (*LCT*, *Bifidobacterium*), was associated with multiple dietary and metabolic phenotypes, and the causal involvement of the SNP across pairs of traits was confirmed by colocalization testing (PP.H4.abf > 0.9) for 49 of 51 tested phenotypes. The NC_000002.11:g.136616754=(rs182549) allele, which predisposes individuals to lactose intolerance, was negatively associated with obesity[33] and positively associated with type 2 diabetes mellitus diagnosis (odds ratio (OR) = 1.057 (95% CI: 1.031, 1.085), $P=1.74\times10^{-5}$), family history of type 2 diabetes mellitus (paternal: OR = 1.054 (95% CI: 1.035, 1.073), $P=1.41\times10^{-8}$; maternal: OR = 1.035 (95% CI: 1.016, 1.053), $P=0.0002$; siblings: OR = 1.03 (95% CI: 1.009, 1.052)) and several nutritional phenotypes in the UK Biobank cohort[32]. Moreover, the functional *LCT* SNP rs4988235 variant is associated with 1,5-anhydroglucitol ($P=4.23\times10^{-28}$)[34], an indicator of glycemic variability[35]. There was a nominally significant genetic correlation (r_g) of *Bifidobacterium* with raw vegetable intake ($r_g=0.36$, $P=0.0016$), but this correlation was not statistically significant after correction for multiple testing.

NC_000019.9:g.49218060=(rs35866622, *FUT1/FUT2* locus) was positively associated with fish intake and height. The secretor allele was negatively associated with the risks of cholelithiasis and Crohn's disease, alcohol intake frequency, high cholesterol and waist-to-hip ratio (adjusted for body mass index (BMI), with PP.H4.abf > 0.9).

Consistent with the single SNP analysis, gene-based PheWAS also showed a strong link between the *LCT* locus and metabolic traits (for example, $P=5.7\times10^{-9}$ for BMI), whereas several factors including nutritional (for example, $P=1.26\times10^{-20}$ for oily fish intake), immune-related (for example, $P=1.73\times10^{-12}$ for mean platelet volume), gastrointestinal (for example, $P=8.77\times10^{-14}$ for

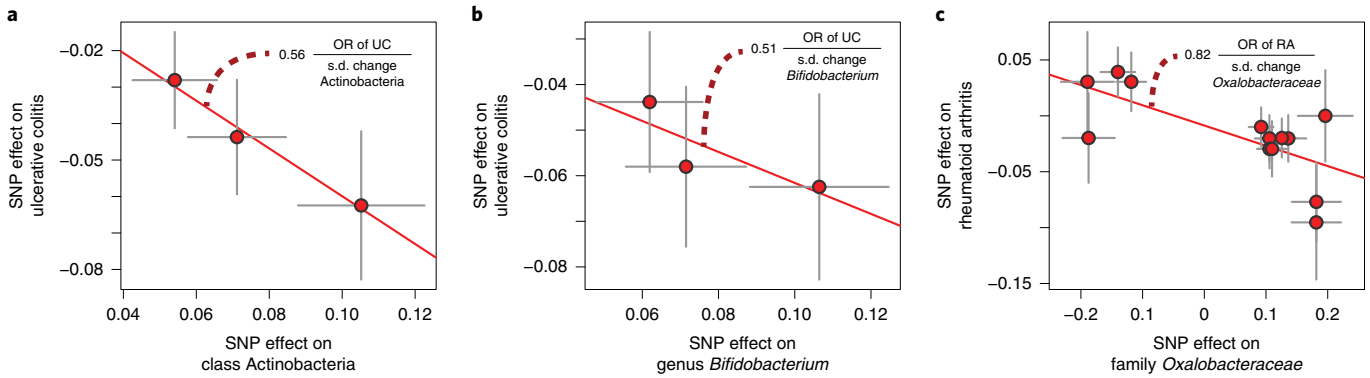


Fig. 6 | Mendelian randomization analysis. The *x* axes show the SNP–exposure effect and the *y* axes show the SNP–outcome effect (SEs denoted as segments). **a**, MR analysis of class Actinobacteria (exposure) and ulcerative colitis (UC; outcome). **b**, MR analysis of genus *Bifidobacterium* (exposure) and ulcerative colitis (outcome). **c**, MR analysis of family Oxalobacteraceae (exposure) and rheumatoid arthritis (RA; outcome).

cholelithiasis) and metabolic signals (for example, $P = 1.13 \times 10^{-13}$ for high cholesterol) mapped to the *FUT1*/*FUT2* locus (Fig. 5 and Supplementary Table 13).

Finally, we performed a phenotype domain enrichment analysis (Methods). We observed that top loci were enriched with signals associated with the metabolic domain supported by four mbTLs, followed by nutritional, cellular, immunological, psychiatric, ophthalmological, respiratory and reproductive traits and the activities domain (Fig. 5 and Supplementary Table 14).

Mendelian randomization analysis. To identify the potential causal links between gut microbial taxa and phenotypes, we performed bidirectional two-sample MR analyses using the TwoSampleMR package[36]. We focused on two groups of phenotypes: diseases (autoimmune, cardiovascular, metabolic and psychiatric) and nutritional phenotypes[37–42]. The complexity of the mechanisms by which host genetics affect microbiome composition, and the limited impact of genetic variants on microbial taxa variability, require caution when performing and interpreting causality estimation using MR analysis[43]. We therefore performed several sensitivity analyses and excluded any results that showed evidence of being confounded by pleiotropy (Methods). Only pairs supported by three or more SNPs were considered. With these strict cutoffs, no evidence for causal relationships between microbiome taxa and dietary preferences was identified (Supplementary Tables 15 and 16). However, our results suggest that a higher abundance of the class Actinobacteria and its genus *Bifidobacterium* may have a protective effect on ulcerative colitis (Actinobacteria: OR = 0.56 (95% CI: 0.44–0.71) for each SD increase in bacterial abundance, Benjamini–Hochberg (BH)-adjusted *P* value for multiple testing $P_{BHadj} = 8.8 \times 10^{-4}$; *Bifidobacterium*: OR = 0.51 (95% CI: 0.39–0.71), $P_{BHadj} = 9.8 \times 10^{-5}$; Fig. 6a,b). We also observed that higher abundance of the family Oxalobacteraceae had a protective effect on rheumatoid arthritis (OR = 0.82 (95% CI: 0.74–0.91), $P_{BHadj} = 0.028$, Fig. 6c).

Discussion

We report here on the relationship between host genetics and gut microbiome composition in 18,340 individuals from 24 population-based cohorts of European, Hispanic, Middle Eastern, Asian and African ancestries. We have estimated the heritability of the human gut microbiome and the effect of host genetics on the presence and abundance of individual microbial taxa. We studied the heterogeneity of the mbTL signals and characterized the impact of technical and biological factors on their effect magnitude. In addition, we explored the relevance of the identified mbTLs to health-related traits using GSEA, PheWAS and MR approaches.

Our large, multiancestry study allowed for an informative investigation of the human gut microbiome. However, there was large heterogeneity in the data, which reflects biological differences across the cohorts and methodological differences in the processing of samples. Overall, seven different methods of fecal DNA extraction and three different 16S rRNA regions were used[12,44]. In addition, differences in the ancestries, ages and BMIs of the participants led to a remarkable variation in microbiome richness, diversity and composition across cohorts. Diet, medication and lifestyle, among other factors[2,3], are known to influence the microbiome but were not included in our analysis because these data were not available for all cohorts. Large variation in the microbiome composition may have reduced the power of our mbTL analysis (Supplementary Note).

We did not detect a host genetic effect on bacterial diversity, in line with a lack of its detectable heritability. Thirty-one taxon-specific mbTLs (20 mbQTLs and 11 mbBTLs) were identified at a *P* value $< 5 \times 10^{-8}$. Even with our large sample size, the number of mbTLs identified is rather modest. Only the association of the *LCT* locus with *Bifidobacterium* ($P = 1.28 \times 10^{-20}$) passed the conservative study-wide significance threshold of $P > 1.95 \times 10^{-10}$. However, we observed that heritable taxa tended to have more genome-wide significant loci and suggestively associated loci, and twin-based heritability was significantly correlated with SNP-based heritability. Our results confirm that only a subset of gut bacteria is heritable, and that the genetic architecture affecting the abundance of heritable taxa is complex and polygenic.

The association between the *LCT* locus and the *Bifidobacterium* genus was the strongest in our study. It has been shown that the functional SNP in the *LCT* locus rs4988235 determines not only the abundance of the *Bifidobacterium* genus but also the strength of the association between this genus and milk/dairy consumption[7]. Here, we showed the ancestry heterogeneity and age-dependent nature of the *LCT* and *Bifidobacterium* association—the effect is weaker in children and adolescents—consistent with existing knowledge on lactose intolerance[45,46]. The strongest mbQTL effect was observed in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) cohort that comprises individuals of Hispanic/Latin American ancestry and shows the highest prevalence of the lactose intolerant NC_000002.11:g.136616754CC (rs182549) genotype (683 of 1,097 individuals).

To explore the potential functional effects of mbTLs on health-related traits, we used GSEA, PheWAS and MR approaches. The GSEA indicated enrichment of mbQTLs for genes expressed in the small intestine and brain. These results support the existence of the gut–brain axis mediated by the microbiome and likely influencing gastrointestinal, brain and mood disorders[47–49]. In addition,

the PheWAS analysis identified a significant overlap between the genetic variants affecting gut microorganisms and a broad range of host characteristics, including psychiatric, metabolic and immunological traits, and nutritional preferences, among other phenotype groups (Supplementary Table 14). Moreover, genetic determinants of bacterial abundance are involved in regulating host metabolism, particularly obesity-related traits. Among the interesting bacteria, earlier studies have linked the relative abundances of *Ruminococcus*[50], *Lachnospiraceae*[51] and *Ruminococcaceae*[52] to obesity. PheWAS analysis also indicated that SNPs from the *LCT* and *FUT2* loci that associated with bacterial taxa are also associated with dietary preference factors, including fish, cereal, bread, alcohol, vegetable and ground coffee intake, along with other dietary phenotypes. Interestingly, other genes found to be associated with mbTLs also included olfactory receptors (*OR1F1*) and genes involved in the absorption and metabolism of vitamins B_2 and B_{12} (*RFK* and *CUBN*).

Genetic anchors to microbiome variation also allow for estimation of causal links with complex traits through MR approaches[53–55]. MR results indicate that Actinobacteria and *Bifidobacterium* might have a protective effect in ulcerative colitis. Cross-sectional studies have reported an increased abundance of Actinobacteria in healthy individuals as compared to patients with inflammatory bowel disease[56,57], although these results have not always been consistent[58,59]. *Bifidobacterium* was also previously shown to have a beneficial effect on ulcerative colitis in a clinical trial[58,60]. We also revealed that abundance of the family Oxalobacteraceae in the gut microbiome might be protective for rheumatoid arthritis; the abundance of this family in lung showed a negative association with rheumatoid arthritis previously[61]. Protective effects of the bacterial taxa on these diseases support the potential of microbiome-based therapy.

To our knowledge, we report the largest study to date investigating the genetics of the human microbiome across multiple ancestries. Microbiome heterogeneity and high interindividual variability substantially reduces the statistical power of microbiome-wide analyses: similar to earlier microbiome GWAS studies, we report a limited number of associated loci. Nevertheless, our results point to causal relationships between specific loci, bacterial taxa and health-related traits. Heritability estimates suggest that these associations are likely part of a larger spectrum that is undetectable in the current study sample size. This warrants future research that should take advantage of larger sample sizes, harmonized protocols and more advanced microbiome analysis methods, including metagenomics sequencing instead of 16S profiling and quantification of bacterial cell counts. Given the essential role of the gut microbiome in the metabolism of food and drugs, our results contribute to the development of personalized nutrition and medication strategies based on both host genomics and microbiome data.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41588-020-00763-1.

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Methods

Data collection. A total of 24 cohorts, comprising 18,340 participants of different ancestries and ages, participated in the microbiome GWAS analysis (Supplementary Tables 1 and 2). The Supplementary Note provides detailed descriptions of data collection for each cohort.

16S microbiome data processing. The rationale behind the selection of the 16S rRNA processing pipeline was described previously[11]. In short, the divergence in the 16S rRNA gene domains between cohorts makes OTU-level analysis impossible, while the use of a direct taxonomic classification of the reads and an up-to-date reference database allowed us to achieve good between-domain concordance of taxonomic composition and a higher mapping rate.

The participating cohorts varied in their sample collection protocol, selection of DNA purification kits used to extract DNA from fecal samples, the 16S domain selected for PCR (Supplementary Table 1), read length and depth, post-sequencing quality control (QC) and the software used to merge tags of paired-end sequencing. After processing the QC-filtered merged reads, all cohorts implemented the standardized 16S processing pipeline (https://github.com/alexa-kur/miQTL_cookbook/) that uses SILVA (release 128)[15] as a reference database, with truncation of the taxonomic resolution of the database to genus level.

Briefly, the procedure was as follows. First, all samples were rarefied to 10,000 reads using a predefined random seed to allow for rarefaction reproducibility. Samples with fewer than 10,000 reads were discarded. Second, RDP classifier (v.2.12)[16] was used to bin the reads to a reference database. For each taxonomic level, the posterior probability of 0.8 was used as a cutoff to bin each read to the corresponding taxon. The posterior cutoff probability was traced for each taxonomic level separately. For example, if the posterior probability passed the cutoff on family level but not on genus level, the read was binned to taxonomy on the family level (all corresponding upper taxonomic levels) and discarded on the genus level. It was also assigned to a special 'NOTAX_genus' pseudo-taxon to maintain data compositionality.

To characterize the contribution of cohort-wise metadata (16S domain, DNA extraction method, cohort ancestry, lysis temperature and type of lysis buffer) to the microbiome composition, we used a distance-based redundancy analysis test in which each cohort represented a sample and variables represented mean abundances of genera in the corresponding cohort (taxa with prevalence below 20% were discarded). The association of metadata with richness was performed by multivariate linear regression analysis.

The alpha diversity indices, including Shannon, Simpson and inverse Simpson indices, were calculated on genus level with non-adjusted, non-transformed taxa counts. For all other analyses, the taxonomic counts of non-zero samples were natural log transformed and adjusted for potential covariate effects using linear regression. The list of covariates used in the regression models varied between cohorts, but always included sex, age, genetic principal components (PCs) calculated on non-imputed genetic data (3 PCs for monoancesty cohorts, 10 PCs for multiancestry cohorts and 5 PCs for the HCHS/SOL cohort as a multiancestry population of different, but closely related ancestries; see Supplementary Note for cohort descriptions) and cohort-specific potential microbiome batch effects, if applicable. Variables such as the length of time in non-frozen storage and the 16S sequencing batch were also included. The residuals of the adjustment were then scaled and centered (mean = 0 and SD = 1).

In the analysis of microbiome composition heterogeneity, the cohorts SHIP/SHIP-TREND and GEM_HCE_v12/GEM_HCE_v24/GEM_HCE_ICHIP were merged to SHIP and GEM, respectively, because they were analyzed with exactly the same protocols in the same laboratories. In the microbiome–genetics analysis, these five cohorts were included individually as they differed in the genotyping arrays and/or general populations they represented.

For each cohort, only the taxa present in more than 10% of the samples were included in the mbQTL mapping, whereas taxa present in more than 10% but less than 90% of the samples were included in the mbBTL mapping (Supplementary Table 3). Study-wide cutoffs for mbQTL mapping included an effective sample size of at least 3,000 samples and presence in at least three cohorts. For mbBTLs, a mean abundance higher than 1% in the taxon-positive samples was required. This resulted in 211 taxa (131 genera, 35 families, 20 orders, 16 classes and 9 phyla) that passed taxon inclusion cutoffs for mbQTL analysis and 177 taxa (108 genera, 34 families, 16 orders, 12 classes and 7 phyla) for mbBTL analysis.

Genetic data processing. Despite the difference in genotyping array platforms, most cohorts used similar procedures for imputation and post-imputation filtering steps. Twenty-three of 24 cohorts used the Michigan Imputation Server (https://imputationserver.sph.umich.edu/index.html) for imputation, using the HRC 1.0 or 1.1 reference panel[62]. Due to restrictions in manipulating data, the PNP study employed an in-house pipeline for imputation instead, using IMPUTE2 software (v.2.3.2)[63,64] and the 1000G reference panel with addition of population-matched genotypes of Jewish individuals[65]. The post-imputation cutoffs were the same for PNP and the other cohorts.

Post-imputation VCFs were transformed into TriTyper format and filtered using GenotypeHarmonizer software (v.1.4.20)[66]. The following cutoffs were

1,009 GWAS performed before the UK Biobank effort, all categorized under 27 phenotype domains. Next, we tested if any of these 27 domains were enriched by the phenotypes associated with one of the SNPs of interest (using a liberal *P*-value threshold of 0.05 for the SNP–phenotype association) as compared to the expected distributions under the null hypothesis. To obtain the distributions under the null hypothesis, we selected the best matching 1,000 SNPs for each top SNP using SNPSNAP[72], matched by allele frequency, gene density, number of LD pairs and distance from the closest gene.

We then extracted corresponding results from the GWASATLAS for the matched 30,000 SNPs (1,000 matching SNPs for each top mbTL SNP). The enrichment of each domain was tested by comparing the proportions of observed and expected significant results for the SNPs of interest using the 'prop.test' function in R. This resulted in one-sided *P* values and ORs. Seven domains (aging; body structures; connective tissue; ear, nose and throat; infection; muscular; and social interactions) that included fewer than 20 GWAS tables were excluded from the enrichment tests, resulting in 20 domains. We used a conservative Bonferroni-based *P*-value threshold of 8.06×10^{-5} for the enrichment testing, accounting for 20 domains and a total of 31 mbTL top SNPs derived from both the mbQTL and mbBTL mapping. In addition, we performed gene-based PheWAS lookups in the GWASATLAS for candidate genes of interest within 250 kb around the association peaks, as defined by the FUMA algorithms.

The genetic correlation between *Bifidobacterium* and its PheWAS-related traits (Supplementary Table 12) was estimated following an LD-score regression approach[67] using the 'ldsc' tool. For testing colocalization of the PheWAS signals, we used the approximate Bayes factor approach as implemented by the 'coloc.abf' function from the 'coloc' library in R[73], using genetic variants within ±250 kb around the top signals.

Mendelian randomization analysis. MR analyses were performed in R using TwoSampleMR package (v.0.5.5)[36]. Causality direction was tested between the microbiome and two data types: (1) autoimmune, cardiovascular, metabolic (including weight-related phenotypes) and psychological diseases (GWAS summary statistics from MRBase[36]) known to be associated with microbiome composition[2,3,37–42,47] and (2) 42 nutritional phenotypes and alcohol intake frequency from the UK Biobank round 2 (http://www.nealelab.is/uk-biobank/).

For MR analyses, the combined meta-analysis effects and s.e. values from inverse-variance meta-analysis were used.

To test if a complex trait affected microbiome composition, we selected independent genetic variants associated with complex traits at the genome-wide significant level ($P < 5 \times 10^{-8}$) and used these as instruments in our MR analyses. For complex diseases, we transformed ORs and CIs to effect sizes and s.e. values using the built-in function of the TwoSampleMR package. To test if microbiome changes were causally linked to complex traits, we first confined ourselves to bacteria with genome-wide significant QTLs. For these, we selected all SNPs with a less stringent cutoff of $P < 1 \times 10^{-5}$ in our MR analyses as instruments. This strategy was used to increase the number of SNPs available to perform sensitivity analyses, as shown previously[53]. Independent SNPs were selected as instrumental variables based on $r^2 < 0.001$ in 1000 G European data, within the TwoSampleMR package. When no shared SNPs were available between exposure and outcome, proxies from the 1000G European data ($r^2 > 0.8$) were added. We kept only the results based on at least three shared SNPs. MR causality tests were performed using the Wald ratio, and Wald ratios were meta-analyzed using the IVW method[74]. We also estimated the causality using additional methods: the weighted mode method[75], which provides an alternative approach to IVW; MR-Egger[76], which estimates the degree of horizontal pleiotropy in the data; and MR PRESSO[77], which estimates the pleiotropy and corrects for it by removing outliers from the IVW model. We also assessed the heterogeneity of the results using Cochran's *Q* statistic[74] and using leave-one-out analyses[36]. We estimated instrument variable strengths using *F* statistics: the amount of variance explained by instrument variables was calculated for each exposure using the TwoSampleMR package (get_r_from_lor function) for binary traits and phenotypic variation (PVE) as defined by Shim et al.[78] for quantitative traits. *F* statistics were then calculated as $\frac{r^2 \times (N-1-k)}{(1-r^2) \times k}$, where r^2 is the variance explained, *N* is the sample size and *k* is the number of instrument variables. We retained the results for the conventional threshold of *F* statistics > 10 (ref. [79]).

After performing the MR tests, we excluded duplicated GWAS traits, as the same phenotype is often studied in multiple GWAS. To remove the duplicates, we kept the study with the largest sample size among all the tested GWAS studies for each trait.

After excluding duplicates and tests performed with weak instruments (*F* statistics < 10), we applied a BH correction for multiple testing to the results obtained from the IVW MR test, and subsequently used a stringent filtering procedure on the significant results to avoid false positives. Specifically, we removed the MR results that were based on fewer than three SNPs and thus could not be further investigated with sensitivity analyses. We also removed the MR results that were not supported by other MR tests (weighted mode method $P > 0.05$, MR PRESSO $P > 0.05$) and those that showed substantial pleiotropy or heterogeneity as estimated by MR-Egger (MR-Egger intercept $P < 0.05$) or MR PRESSO outliers-adjusted test ($P > 0.05$), as well as those where leave-one-out

applied for inclusion: minor allele frequency > 0.05, pointwise imputation QC > 0.4 and SNP-wise call rate filtering > 0.95.

Heritability analysis. Heritability was calculated using data collected on 169 MZ and 419 DZ pairs of twins from the TwinsUK cohort (total of 1,176 individuals). Twin-based heritability was calculated by fitting an ACE model using the OpenMx package (v.2.8.3), as previously described[5]. Before heritability estimation, the taxonomic abundance was normalized using inverse rank-sum transformation. Since the NTR cohort comprised only MZ twins, the between-cohort heritability concordance was calculated as the correlation of ICC for MZ twins. The Pearson correlation of ICC between the TwinsUK and NTR cohorts was used to estimate the concordance. For mbQTLs, SNP-based heritability was calculated by LD score regression using the 'LDSC' tool[67].

Microbiome GWAS analysis. The modified version of the eQTL mapping pipeline (https://github.com/molgenis/systemsgenetics/tree/master/eqtl-mapping-pipeline/) was used to perform mbQTL mapping[18].

The microbiome GWAS was performed in three ways. First, we performed GWAS on three microbiome alpha diversity metrics (Shannon, Simpson and Inverse Simpson), using the Spearman correlation between SNP dosages and alpha diversity metrics after adjustment for age, sex, technical covariates and genetic PCs.

Second, we used the Spearman correlation to identify loci that affected the covariate-adjusted abundance of bacterial taxa, excluding samples with zero abundance (mbQTLs).

Third, we identified the loci associated with probability of presence versus absence of the bacterial taxon (mbBTLs). To perform mbBTL analysis, we used a two-stage approach composed of fast correlation screening followed by logistic regression analysis as a robust method for binary traits GWAS[19]. First, we calculated the Pearson correlation between SNP dosage and bacterial presence encoded as 0/1, without adjusting for any covariate effect and using the previously mentioned eQTL mapping pipeline, and used weighted *z*-score meta-analysis to calculate noncentrality for SNP–taxon association. Finally, all SNP–taxon pairs with a *P* value $<1\times10^{-4}$ in the first-stage meta-analysis were recalculated using multiple logistic regression (R base package, versions from 3.2.0 to 3.5.1 depending on the group) with bacterial presence as an outcome and using SNP dosage along with the list of covariates as predictors. All the mbBTLs that reached the nominal genome-wide significance threshold ($P<5\times10^{-8}$) in logistic regression had a Pearson correlation *P* value (at first stage) more significant than $P<10^{-6}$, presuming the completeness of the two-stage procedure in revealing genome-wide significant mbBTL using a cutoff of $P<10^{-4}$ at the first stage of analysis.

mbTL meta-analysis. Meta-analysis was performed using a weighted *z*-score method implemented in BinaryMetaAnalyzer (v.1.0.13B available on MiBioGen Cookbook), a part of the eQTL mapping pipeline that was used in large-scale eQTL meta-analyses[18,20]. For each cohort, *z*-scores were calculated from Spearman correlation *P* values using inverse normal transformation, transforming two-tailed *P* values to one-tailed *P* values and tracing the effect directions using the following formula:

$$\mathrm{sign}(R_{Sp})\times \mathrm{qnorm}(1-P/2)$$

where $\mathrm{sign}(R_{Sp})$ denotes the sign of Spearman correlation, 'qnorm' denotes the quantile function for the normal distribution and *P* denotes the two-tailed *P* value of the Spearman correlation. For mbQTLs, the cohorts were weighted by the square root of the effective sample size (the number of samples having the bacterial taxon). For mbQTLs, the square root of the reported cohort size was used as a weighting for each study. The summary statistics generated for mbQTLs also include meta-analysis effect sizes and s.e. values. These were generated using the inverse-variance weighted (IVW) meta-analysis method performed on the per-cohort effect sizes and standard errors, backtracked from association *z*-scores and minor allele frequencies using the strategy proposed and implemented by Zhu et al.[68], who also provide a detailed derivation of the following equations:

$$\hat{b} = zS$$

$$S = \frac{1}{\sqrt{2p(1-p)(n+z^2)}}$$

where *b* is the estimated effect size, *S* is the estimated s.e., *p* is the allele frequency and *n* is the sample size.

Heterogeneity exploration analysis. Cross-study heterogeneity of the effects of genetic variants in the relative abundance of taxonomical units was assessed using Cochran's *Q* test for heterogeneity[69], as implemented in METAL (v2018-08-28)[70], for all genome-wide significant variants ($P<5\times10^{-8}$) found in our main analysis. To avoid reporting false-positive associations due to different study designs or data collection methods, we used a stringent threshold of $P<0.05$ to reject the null hypothesis of no heterogeneity. This threshold is conservative considering that several variants were tested simultaneously, and no correction for multiple testing was applied. When there was evidence of heterogeneity, a random-effects model was also implemented at the meta-analysis level to confirm the association results, using the metaphor R package (v.2.0-0; https://cran.r-project.org/web/packages/metafor/metafor.pdf).

Additionally, when there was evidence for heterogeneity of a SNP effect across cohorts, we implemented a meta-regression approach using the same package to assess whether variables such as age, ancestry or sequenced region could explain the observed effect-size heterogeneity.

Analysis of SNP–age interaction analysis in the *LCT* locus. To discover whether the association of functional SNPs in the *LCT* locus to the abundance of the *Bifidobacterium* genus varied between groups of adults and infants, we performed age–SNP interaction analysis in the GEM cohort, which comprises three subcohorts that each have a comparable number of individuals above and below pubertal age. The age of 17 years was selected to split the cohort into two groups: adolescents or adults. Since the GEM cohort was composed of three subcohorts of different ancestry composition, we evaluated the interaction in both joint analysis and in each subcohort separately, using the following formula:

$$\begin{aligned}\mathrm{Bac} = \ & \mathrm{sex} + \mathrm{PC}(1-3) + \mathrm{age}_{\mathrm{group}} + \mathrm{cohort} + \mathrm{SNP}_{\mathrm{dos}} + \mathrm{SNP}_{\mathrm{HZ}} \\ & + \mathrm{SNP}_{\mathrm{GT}} : \mathrm{age}_{\mathrm{group}}\end{aligned}$$

where 'bac' is the log-transformed count of genus *Bifidobacterium*, 'PC(1–3)' are three floats with the first three genetic PCs, 'cohort' is a batch variable that determines the cohort to which the sample belongs, '$\mathrm{SNP}_{\mathrm{dos}}$' is a float-encoded dosage of alternative allele, '$\mathrm{SNP}_{\mathrm{HZ}}$' is a Boolean variable describing heterozygosity, '$\mathrm{SNP}_{\mathrm{GT}}$' is a genotype encoded as an unordered factor and '$\mathrm{age}_{\mathrm{group}}$' is a two-level factor (above or below split level). The inclusion of a numeric dosage variable and a Boolean $\mathrm{SNP}_{\mathrm{HZ}}$ variable allowed us to properly adjust for the recessive effect of the SNP on *Bifidobacterium* abundance without neglecting SNP imputation uncertainty as embedded in SNP dosage.

The analysis was then repeated for each GEM subcohort separately, using the same model.

Association of mbTL-associated taxa with host phenotypes. Bacterial taxa found to be significantly associated with genetic determinants were correlated with 207 host phenotypes, including the intrinsic host properties, diet, disease and medication information, in the LLD and FGFP cohorts. We used the Spearman correlation with BH adjustment for multiple testing to assess the correlation between phenotypes and bacteria that had mbQTLs. For the taxa with mbQTLs, samples with zero abundance were truncated. For the taxa with mbBTLs, the abundance was transformed to a binary trait encoding presence/absence.

FUMA analyses of meta-analysis results. Functional mapping and annotation of 31 meta-analysis results were performed with FUMA (v1.3.5), an integrated web-based platform[31]. Summary statistics from the mbQTL analyses for each of the 20 independent association signals were used in the analysis. Genome-wide significant loci and their boundaries were defined as nonoverlapping genomic regions that extend across an LD window of $r^2 \geq 0.4$ (based on the 1000G European reference panel)[71] from the association signals with $P<5.0\times10^{-8}$. Independent ($r^2<0.1$) lead SNPs from each locus were defined as those most strongly associated with a microbial trait (that is, with the lowest *P* value) at the specific region. Multiple risk loci were merged into a single genomic locus if the distance between their LD blocks was <250 kb.

Functional annotation of all candidate risk SNPs was obtained from different repositories integrated in FUMA. Furthermore, these functionally annotated SNPs were mapped to protein-coding genes using the following two strategies: (1) positional mapping, with the maximum distance of 10 kb to protein-coding genes and (2) eQTL mapping, using information from data repositories such as GTEx v7 and Blood eQTL browser (http://genenetwork.nl/bloodeqtlbrowser/)[20].

As the mbBTL mapping procedure provided accurate statistics for only a subset of SNPs ('Microbiome GWAS analysis'), and we thus lacked full summary statistics, we only performed positional mapping for mbBTLs, taking in the protein-coding genes within a 10-kb distance of the ten leading SNPs for each trait.

All mapped protein-coding genes were combined into one list for either mbQTL or mbBTL analysis before performing GSEA integrated in FUMA. In further investigations, hypergeometric tests of enrichment of all mapped genes were performed not only in tissue-specific (differentially expressed) gene sets, but also in gene sets curated from various sources, for example, MsigDB. We reported all enriched gene sets (≥2) with a false discovery rate -adjusted *P* value < 0.05.

PheWAS, genetic correlation and colocalization analysis. We performed the PheWAS lookups in the summary statistics results of 4,155 traits collected by the GWASATLAS[32] (http://atlas.ctglab.nl/, accessed on 25 September 2019) database for the top SNPs for each mbQTL locus that were revealed by either mbQTL or mbBTL mapping. GWASATLAS includes 600 traits from the UK Biobank and is enriched with extensive phenotypes on proteomics ($n=1,124$ proteins), hematology ($n=36$), metabolomics ($n=1,145$ metabolic features) and immune markers ($n=241$), studied across variable sample sizes. It also contains

analysis identified one SNP driving the signal (all but one leave-one-out configurations had $P<0.05$). Of note, the MR-Egger slope, which represents the causal estimate, was not used as a filtering step given the reduced power to detect causal effects. Furthermore, for all but one of the reported MR results that passed all the filters above, the MR-Egger slope P value was greater than 0.05; therefore, an MR-Egger intercept $P<0.05$ cannot be used to exclude the presence of pleiotropy. Even though many of our MR-Egger intercept results provided little evidence of directional pleiotropy, it is worth noting that a $P<0.05$ cannot exclude the presence of pleiotropy and requires further understanding of the biological mechanisms underpinning the relationship between genetic variation, the gut microbiome and health outcomes. To exclude more complex causality scenarios, we also removed those results for which the reverse MR P value was below 0.05. Of note, the causal relationship identified for the microbiome feature class Actinobacteria (as exposure) and ulcerative colitis (outcome) showed a consistent effect direction when just using the only genome-wide significant SNP, but with wider CIs (OR = 0.40 (95% CI: 0.22–0.71), $P_{nominal}=0.002$).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Full GWAS summary statistics for mbQTLs are available at www.mibiogen.org, built using the MOLGENIS framework[80].
16S data availability:
BSPSPC and FOCUS data is available from the Sequence Read Archive (SRA) under accession PRJNA673102.
All CARDIA data, including 16S rRNA sequencing, cannot be made publicly available due to the confidentiality restrictions. The data can be requested from CARDIA Study Data Coordinating Center at the University of Alabama at Birmingham, following CARDIA Confidentiality Certification rules. The process for obtaining data through CARDIA is outlined at https://www.cardia.dopm.uab.edu/publications-2/publications-documents.
COPSAC data are available on SRA (PRJNA683912).
DanFunD data are not deposited on the public databases due to legal and ethical restrictions. Access to the data and biological material can be granted by the DanFunD steering committee (https://www.frederiksberghospital.dk/ckff/sektioner/SBE/danfund/Sider/How-to-collaborate.aspx).
FGFP data are available on the European Genome-Phenome Archive (EGA) under accession EGAS00001004420.
GEM data are available on the SRA (PRJEB14839).
Generation R and Rotterdam Study data cannot be made publicly available due to ethical and legal restrictions; these data are available upon request to the data manager of the Rotterdam Study (f.vanrooij@erasmusmc.nl) or of the Generation R Study (c.kruithof@erasmusmc.nl), subject to local rules and regulations.
HCHS/SOL data are available from the European Nucleotide Archive (ENA) under accession ERP117287.
KSCS data are available at the public repository, Clinical and Omics data archives in the Korea National Institute of Health under accession R000635.
LLD and MIBS data are available from EGA (EGAS00001001704 and EGAS0000100924).
METSIM data are available on the SRA (SRP097785).
NGRC data are available on the ENA (ERP016332).
The NTR has a data access committee that reviews data requests and will make data available to interested researchers. The data come from extended twin families and pedigree structures with twins, which create privacy concerns and thus cannot be shared on publicly available databases. Researchers may contact eco.de.geus@vu.nl for data requests.
PNP is available on the ENA (PRJEB11532).
POPCOL is available on the EGA (EGAS00001004869).
SHIP and SHIP-TREND data can be obtained from the SHIP data management unit via an online data access application form (https://www.fvcm.med.uni-greifswald.de/dd_service/data_use_intro.php).
TwinsUK data are available on the ENA under accession ERP015317.

Code availability

All code used in the study is available on the Consortium GitHub (https://github.com/alexa-kur/miQTL_cookbook) or on the websites of corresponding software packages.

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Author contributions

A.K., A.Z., R. Kraaijr, C.M.-G., L.F. and J.R. conceived and designed the study. A.K., C.M.-G., R.B., D.R. and J.W. were responsible for coordinating and performing meta-analysis. A.D., C.L.R., J.A.R.G., C.T.F., X.L., D.Z. and M.J.B. led the specific downstream analyses and should be considered as shared second authors. Specifically, A.D. performed the PheWAS analysis, C.L.R. and C.T.F. performed the heritability analysis in TwinsUK and NTR cohorts, respectively, and J.A.R.G performed the age-related analysis of the *LCT* locus. X.L. ran and interpreted the FUMA analysis, and D.Z. ran and interpreted the MR analysis. M.J.B. substantially contributed to the development of the analysis pipeline and protocols. R.K., J.R. and A.Z. jointly supervised the project. A.v.d.G., A.C., H.-J.W., Urmo V., M.J.B., S.S. and L.F. developed the pipeline for the meta-analysis and contributed to the methodology and statistical analysis. K.W. contributed to the PheWAS enrichment analysis. A.K., C.M.-G., R.B., D.R., J.W., A.D., C.L.R., J.A.R.G., C.T.F., X.L., D.Z., M.J.B., M.D.A., S.S., R. Kraaij, J.R. and A.Z. wrote the manuscript, with contributions from all authors. K.A.M., L.J.L. and M.F. collected and managed the CARDIA cohort. A.D.P., J.A.R.G., K.C., L.B. and W.T. collected and managed the GEM cohort. H.B., J.S., J.T., S.A.S. and S.J.S collected and managed the COPSAC study. D.B., O.P., T.H., T.J. and T.H.H. collected and managed the DanFunD study. D.A.H., G.F., J.R., J.W., K.H.W., M.J., N.J.T., R.Y.T., R.B. and S.V.-S. collected, genotyped and managed the FGFP study. C.M.-G., F.R., H.A.M., L.D. and V.W.V.J. collected and managed the Generation R study. H.-N.K., H.S. and H.-L.K. collected and managed the KSCS study. C.W., J.F., A.Z., L.F., S.S. and A.K. collected and managed the LLD cohort. A.J.L., E.O., K.L., M. Laaksok and M.B. collected and managed the METSIM cohort. A.A.M.M., D.M.A.E.J., D.K. and Z.M. collected and managed the MIBS-CO cohort. H.P. and Z.D.W. collected and managed the NGRC cohort. C.T.F., D.I.B., E.J.C.G., G.E.D., G.W. and R.G.I. collected and managed the NTR cohort. D. Rothschild, E.B., E.S. and O.W. collected and managed the PNP cohort. A.A., L.A., M.D.A., S. Walter and X.L. collected and managed the PopCol cohort. A.F., C.B., M.C.R., M. Laudes and W.L. collected and managed the BSPSPC and FOCUS cohorts. A.G.U., C.Mv.D, D. Radjabzadeh and R. Kraaij collected and managed the RS cohort data. F.F., F.U.W., G.H., H.V., M.M.L., S. Weiss and U. Völker collected and managed the SHIP and TREND cohorts. L.Y.M., Q.Q., R. Knight, R.C.K. and R.D.B collected and managed the

SOL cohort. C.I.L.R, C.J.S., J.T.B., M.A.J. and T.D.S. collected and managed the TwinsUK cohort. A.A.V. and J.S.-T. contributed to the discussion. All authors approved the final manuscript.

Competing interests

All authors declare no competing interests.

Additional information

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	no software used for data collection
Data analysis	Partly, the software used in the analysis can be found on consortium GitHub: https://github.com/alexa-kur/miQTL_cookbook specific steps of analysis: 16S data processing: RDP classifier v.2.12 (https://github.com/rdpstaff/classifier) with database SILVA v.128 (version prepared for RDP classifier in on Consortium GitHub). Genetics data processing: GenotypeHarmonizer v.1.4.20 on Consortium GitHub IMPUTE2 v.2.3.2 (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html) both mapping pipeline and meta-analyzer are the parts of Molgenis SystemGenetics pipeline (https://github.com/molgenis/systemsgenetics). Specific versions used in the analysis: mbQTL mapping: eQTL-mapping-pipeline v1.4, on Consortium GitHub, meta-analysis: BinaryMetaAnalyzer v.1.0.13B, on Consortium GitHub, Heterogeneity analysis: METAL v.2018-08-28 (http://csg.sph.umich.edu/abecasis/metal/) and R package metafor v.2.0-0 (https://cran.rproject.org/web/packages/metafor/) FUMA and GSEA analysis: FUMA v.1.3.5, https://fuma.ctglab.nl/ PheWAS analysis: https://atlas.ctglab.nl/, accessed 25.09.2019 MR analysis: R package TwoSampleMR v0.4.26 (https://mrcieu.github.io/TwoSampleMR/)

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Full GWAS summary statistics for mbQTLs are available at www.mibiogen.org website built using the MOLGENIS framework.
16S data availability:
BSPSPC and FOCUS data is available from Sequence Read Archive (SRA), PRJNA673102
All CARDIA data, including 16S rRNA sequencing, cannot be made available on publicly available databases due to the confidentiality restrictions. The data can be requested from CARDIA Study Data Coordinating Center at the University of Alabama at Birmingham, following CARDIA Confidentiality Certification rules. The process for obtaining data through CARDIA is outlined at: https://www.cardia.dopm.uab.edu/publications-2/publications-documents.
COPSAC data is available on SRA (PRJNA683912).
DanFunD is not deposited on the public databases due to the legal and ethical restrictions. Access to the data and biological material can be granted by the DanFunD steering committee (https://www.frederiksberghospital.dk/ckff/sektioner/SBE/danfund/Sider/How-to-collaborate.aspx).
FGFP data is available on European Genome-Phenome Archive (EGA), EGAS00001004420
GEM data is available on SRA (PRJEB14839).
Generation R and Rotterdam Study data cannot be made publicly available due to ethical and legal restrictions; these data are available upon request to the data manager of the Rotterdam Study Frank van Rooij (f.vanrooij@erasmusmc.nl) or of the Generation R Study Claudia Kruithof (c.kruithof@erasmusmc.nl) and subject to local rules and regulations.
HCHS/SOL data is available from ENA (European Nucleotide Archive), ERP117287.
KSCS data is available at the public repository, Clinical and Omics data archives (CODA) in the Korea National Institute of Health by accession number R000635 (http://coda.nih.go.kr/coda/coda/search/omics/genome/selectSearchOmicsGenomePop/R000635.do).
LLD and MIBS data are available from EGA, EGAS00001001704, EGAS0000100924).
METSIM data is available on SRA (SRP097785).
NGRC data is available on ENA (ERP016332).
NTR has a data access committee that reviews data requests and will make data available to interested researchers. The data come from extended twin families and pedigree structures with twins, which create privacy concerns and thus cannot be shared on publicly available databases. Researchers may contact prof Eco de Geus (eco.de.geus@vu.nl) for data request..
PNP is available on ENA (PRJEB11532).
POPCOL is available on EGA (EGAS00001004869).
SHIP and SHIP-TREND data can be obtained from the SHIP data management unit and can be applied for online through a data access application form (https://www.fvcm.med.uni-greifswald.de/dd_service/data_use_intro.php)
TwinsUK data is available on the European Nucleotide Archive (ENA, accession ERP015317).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample size of 18,473 samples used is a total sample size from participaing cohorts, after data exclusion criteria. No prior sample size calculations were performed, thus it was determined by the data availability at the moment of the study initiation
Data exclusions	The genetics exclusion criteria for the study is the following: (a) ethnic outliers in monoethnic cohorts to avoid false positives driven by outliers; (b) random selection of one individual from the related group (i.e. MZ or DZ twins), if applicable, to make possible the use of GWAS method similar across cohorts, which doesn't allow to stratify for family structure. For microbiome data, individuals with lower than 10,000 16S reads were excluded; this cutoff is assumed to provide an accuracy in estimating 16S taxonomic profiles, up to genus level resolution.
Replication	Due to the limited power of analysis, no split to discovery/replication group was applied
Randomization	Given the population-based study design, there was no separation to groups in the study
Blinding	there was no blinding during the sample collection from the cohorts, since the majority of cohorts utilize population-representative design

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Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	BSPSPC (PopGen) The PopGen cohort (mean age 61.5 (16.6), 55% male) is a population-based cohort from the area around Kiel, Schleswig-Holstein, Germany. CARDIA (Coronary Artery Risk Development in Young Adults Study) Coronary Artery Risk Development in Young Adults Study (CARDIA) is a population-based prospective study of the evolution of cardiometabolic disease. African American and European American adults were recruited from four U.S. urban areas (Birmingham, AL; Chicago, IL; Minneapolis, MN; Oakland, CA in 1985-1986) (n=5,115, aged 18-30). They have subsequently been examined nine times. A microbiome study was initiated at the Year 30 follow-up examination (2015-2016) in a subset of participants (n=615) who had not taken antibiotics in the past month. Fecal DNA was extracted with the MoBio PowerSoil kit, and the V3-V4 region of the 16S rRNA gene was sequenced with Illumina MiSeq (2x300bp) at HudsonAlpha Institute for Biotechnology (Huntsville, AL, USA). A subset of cohort participants has been genotyped with the Affymetrix Genome-Wide Human SNP Array 6.0. After quality control and removal of participants with non-overlapping data on microbiome and host genetics, data from 114 African Americans and 257 European Americans (total n=371) were available for analysis. NeuroIMAGE+COMPULS NeuroIMAGE+COMPULS is a cohort consisting of two studies, NeuroIMAGEII and COMPULS, and includes participants of Dutch ethnicity. The cohort represents a combination of adults/adolescents/children diagnosed with ADHD and healthy controls. The overlap between samples with genotyping and microbial 16S sequencing data yielded 133 samples (57 females, 76 males, 17(5) years old) for use in the microbiome GWAS analysis. COPSAC2010 The Copenhagen Prospective Studies on Asthma in Childhood 2010 (COPSAC2010) cohort is a prospective mother-child cohort of 700 children and their families, recruited during week 24 of pregnancy, with written informed consent obtained from all mothers. The participants reside in and around Copenhagen, Denmark. The design builds upon the previous COPSAC2000 cohort and is based on detailed longitudinal clinical assessments of asthma, allergy, eczema and other outcomes. At the latest timepoint, we had both genotype and microbiome data for 380 children to include in this study, 73 of whom had taken antibiotics in the six months before the fecal sample date. DanFunD (The Danish study of Functional Disorders) DanFunD is a population-based cohort initiated to outline the epidemiology of functional somatic syndromes. The study population comprises a random sample of 9,656 men and women aged 18-76 years from the general population who were examined from 2011 to 2015. Genotyping using the Human OmniExpress Bead Array (Illumina Inc., San Diego, CA, USA) was conducted on human leukocyte DNA for the entire cohort. A subset of 2,464 participants volunteered to provide a fecal sample collected under standardized conditions. In total, 2,396 samples passed the QC for genotyping and 16S sequencing and were included in the GWAS. FGFP (Flemish Gut Flora Project) The FGFP is a population-based study cohort of 2,482 individuals from the Flanders region of Belgium. Blood and stool samples of volunteers were collected between June 2013 and April 2016After quality control, 2,259 samples had genotype and 16S data (1,328 females, 896 males, mean age 52.3 yrs). FOCUS The FoCus cohort (mean age 51.4(14.6) yrs, 42% male) is a population-based cohort from the area around Kiel, Schleswig-Holstein, Germany, and part of the competence network Food Chain Plus (FoCus, http://www.focus.uni-kiel.de/component/content/article/88.html). GEM (The CCC GEM project) The CCC GEM project is a prospective international research study that is designed to identify the potential triggers that contribute to the onset of Crohn's Disease. Since 2008, the GEM project has recruited over 5,000 healthy first-degree relatives of Crohn's Disease patients with an age range of 6-35 years. We used data from participants recruited in Canada (n=1,115), United States (n=17) and Israel (n=111). Stool DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The V4 hypervariable region of bacterial 16S ribosomal RNA (16S rRNA) was sequenced using a MiSeq platform (Illumina Inc. San Diego, CA, USA) and primers 515F/806R90. The genotyping of the cohort was performed using the HumanCoreEXOME-12v1.1 chip (n=379), HumanCoreEXOME-24v1.0 chip (n=203) and both ImmunoChip and HumanCoreEXOME-12v1.1 chip (n=662) (Illumina, Inc. San Diego, CA, USA). Thus in mbQTL mapping the cohort was split into subcohorts GEM_v12, GEM_v24 and GEM_ICHIP respectively. Among subcohorts, GEM_v24 mostly coprises individuals of Israel ethnicity (70%), while other two

subcohorts are of a European ancestry. Only the sample from one member from each family enrolled in the project was included
in the current microbiome GWAS study. The overlap between samples with genotyping and microbial 16S sequencing data yielded 1,243 samples (676 females, 567 males, median age = 19.0(8.03) yrs) for use in the microbiome GWAS analysis. None had used antibiotic in the three months before fecal collection.
The Generation R Study
The Generation R Study (GenR) is a population-based, prospective, multi-ethnic pregnancy cohort study from fetal life until young adulthood. It is conducted in the city of Rotterdam, the Netherlands91. After stringent quality control, the overlap between samples with genotyping and microbial 16S sequencing data yielded 1,328 samples (656 females, 672 males, mean age
9.8(0.3) years) for use in the microbiome GWAS analysis. None had used antibiotics in the six months before fecal collection.
KSCS (Kangbuk Samsung Cohort Study)
The Kangbuk Samsung Cohort Study (KSCS) is a prospective cohort study to evaluate the natural history, prognosis, and genetic
and environmental determinants of a wide range of health traits and diseases among Korean adults. After quality control, 811 samples (319 females, 492 males, mean age 44.1 yrs) with overlapping genotype and 16S data were included in the microbiome
GWAS.
LifeLines-DEEP (LLD)
The LifeLines-DEEP cohort (LLD) is a subcohort of the prospective LifeLines cohort from the northern provinces of the Netherlands (Groningen, Drenthe and Friesland) and includes participant of Dutch ethnicity. The overlap between samples with
genotyping and microbial 16S sequencing data yielded 875 samples (504 females, 371 males, mean age 45.4(13.3) yrs) used for
the microbiome GWAS analysis, of these 70 participants were PPI users and eight people used antibiotics in the six months previous to fecal collection.
METSIM (METabolic Syndrome In Men)
METSIM
The METabolic Syndrome In Men (METSIM) cohort is a longitudinal population-based cross-sectional cohort comprising of 10,197 randomly selected non-diabetic Finnish men (aged from 45 to 73 years) who were examined in 2005-2010. For the current microbiome GWAS study, we used a subset of the METSIM cohort consisting of 522 samples (mean age 61.91 (5.42) yrs)
with overlapping genotyping and microbial 16S sequencing data. For the current microbiome GWAS study, we used a subset of the METSIM cohort consisting of 522 samples (mean age 61.91 (5.42) yrs) with overlapping genotyping and microbial 16S sequencing data.
MIBS (Maastricht Irritable Bowel Syndrome)
The MIBS cohort with biobank aims to identify subgroups of IBS according to phenotypical and genotypical characterization. At present, it includes 520 subjects with a clinical diagnosis of IBS according to the Rome III criteria (from primary-tertiary care) and 220 age- and gender-matched healthy controls. For the present microbiome GWAS study, only controls (N=80, mean age 48.7(18.2), 43% male) were included.
NGRC (NeuroGenetics Research Consortium)
The NeuroGenetics Research Consortium (NGRC) is a collaborative study of gene-environment-microbiome interaction on Parkinson's disease (PD). It is being conducted in the United States. For the microbiome GWAS study, only 133 control participants were used; they were free of neurodegenerative disease at a mean age of 71.9(7.5) years old, 58% were female.

NTR (the Netherlands Twin Registry)
The NTR collects data and biological samples on Dutch multiples and their family members. One of each twin pair was randomly selected for inclusion in the GWAS analyses (156 twin pairs, 123 unrelated individuals, 279 individuals total, mean age 35.4(12), 29.8% male). Both MZ twins were included for the ICC calculations between MZ twin pairs for comparison with heritability estimates (156 twin pairs). None of the participants reported using antibiotics within six months of fecal collection.
PNP (Personalized Nutrition Project)
The PNP is a large-scale nutrition initiative in Israel that aims to help people make food choices that would normalize their blood glucose level and improve their health and well-being. The cohort has over 1,000 healthy individuals of Israeli ethnicity living in Israel and aged between 18 and 70 years. The cohort consists of self-reported Ashkenazi (n=508), North African (n=64), Middle Eastern (n=34), Sephardi (n=19), Yemenite (n=13) and 'admixed/other' (n=408) ancestries. 481 individuals were included in the current study (mean age 43.7(13.1), 36.4% male).
PopCol (Population-based Colonoscopy)
Population-based Colonoscopy (PopCol) is a cohort study in Stockholm, Sweden, which includes a data-rich set of individuals with data available from bowel symptoms questionnaires, gastroenterology visits, and biospecimensAfter data merging and quality control, we used data from 134 individuals (83 females, 51 males, mean age 54.8(11.3) yrs) in the microbiome-GWAS. Of
these, 6 PopCol participants were proton pump inhibitors (PPI) users and 12 used antibiotics.
Rotterdam Study III
The Rotterdam Study (RS) is a prospective population-based cohort study established in 1990 to study determinants of disease
and disability in Dutch adult/elderly individuals, aged ≥ 40 years. The overlap between samples with genotyping and microbial 16S sequencing data yielded 1,220 samples (705 females, 515 males, mean age 57(5.9) yrs) for use in the microbiome GWAS analysis. Of these 260 participants used PPI, and none used antibiotics in the six months before fecal collection.
SHIP (Study of Health in Pomerania)
The Study of Health in Pomerania (SHIP) is a prospective longitudinal population-based cohort study encompassing two independent cohorts SHIP (N=4,308; baseline examinations 1997-2001) and SHIP-TREND (N=4,420; baseline examinations 2008 -
2012) 1,901 datasets (1,043 females, 858 males, age 53.7(14.0) yrs) with overlapping genotype and microbiome data were included in the current study. Of these, 149 individuals used PPIs and 25 had antibiotics at the time of inclusion.
HCHS/SOL

The Hispanic Community Health Study/Study of Latinos (HCHS/SOL) is a prospective, population-based cohort study of 16,415 Hispanics/Latino adults (ages 18-74 years) who were selected using a two-stage probability sampling design from four US communities (Chicago, IL; Miami, FL; Bronx, NY; San Diego, CA)102,103. The overlap between genetically unrelated subjects with
microbial 16S sequencing data yielded 1,097 samples (676 females, 421 males, age 57.2(10.9) yrs) used in the microbiome GWAS analysis. Of these, 341 used medication including PPIs for indigestion, heartburn, or stomach problems, and 321 used antibiotics in the six months before the fecal collection.
TwinsUK
TwinsUK is a population-based cohort established in 1992 to study the genetic and environmental basis of a range of complex diseases and conditions in adult/elderly twins from the UK One twin out of each pair was randomly excluded from the population
of 1,793 individuals, leaving 1,205 volunteers (1,101 females and 104 males, age 61.5(10.7) yrs) on which to conduct the microbiome GWAS analysis. Of these, 78 used PPIs and 62 had antibiotics 6 months prior to sampling.

Recruitment

The following bias might occur in extrapolation the results to the general population:
1. Despite the multi-ethnic setup of the study, the consortium sampling is still dominated by the cohorts of European ancestry and European residence.
2. 16S taxonomic profiling method used in the study is known to introduce bias in the microbiome composition. There is a significant imbalance in the cohorts methodology in selection of 16S domains and DNA extraction methods, which might lead to underestimate the genetic effects on bacterial taxa which are not sufficiently covered by the methods used by numerous cohorts.
3. Several cohorts participating in the study utilize age-, sex- and symptom-dependent bias in recruitment process.

Ethics oversight

All participants enrolled had signed the informed consent. For LLD/MIBS cohorts approved as clinical studies, only population controls were used in the current analysis.

BSPSPC: approved by the institutional ethical review committee of Kiel University, Germany
CARDIA: approved by Institutional Review Boards of University of Alabama at Birmingham, Birmigham, AL, Kaiser Permanente Division of Research, Oakland CA, University of Minnesota, Minneapolis, MN, and Northwestern University, Chicago, IL.
NeuroIMAGE+COMPULS: approved by the regional ethics committee of each site (Nijmegen and Utrecht: Commissie Mensgebonden Onderzoek Regio Arnhem-Nijmegen, 2013, NL nr 42004.091.12
COPSAC: approved by Danish Ethics Committee (H-B-2008-093) and the Danish Data Protection Agency (2008-41-2599)
DanFunD: approved by Ethical Committee of Copenhagen County (Ethics Committee: KA-2006-0011; H-3-2011-081; H-3-2012-0015) and the Danish Data Protection Agency
FGFP: approved by the medical ethics committee of the University of Brussels–Brussels University Hospital (approval 143201215505, 5/12/2012).
FOCUS: approved by the institutional ethical review committee of Kiel University
GEM: approved by Mount Sinai Hospital Research Ethics Board (Toronto–Managing Center) and local centers
GenerationR: approved by the Medical Ethical Committee of Erasmus MC, University Medical Center Rotterdam.
KSCS: approved by EUMC review board 2014-06-024 and KBSMC review board 2013-01-245.
LLD: Each participant signed an informed consent form before participation in the cohort according to the UMCG Institutional Review Board (IRB; #M12.113965).
METSIM: approved by Ethics Committee of the Northern Savo Hospital District, Finland
MIBS: Each participant signed an informed consent form before participation in the cohort according to the Maastricht University Medical Center (MUMC+) IRB (#MEC 08-2.066.7/pl).
NGRC: approved by institutional review boards at the participating institutions: Albany Medical Center, Emory University, Kaiser
Permanente Northwest Division, New York State Department of Health, Oregon Health & Sciences University (OHSU) and the Department of Veterans Affairs VA Puget Sound Health Care System (VAPSHCS).
NTR: approvCentral Ethics Committee on Research involving human subjects of the VU University Medical Center, Amsterdam
PNP: Approved by Tel Aviv Sourasky Medical Center Institutional Review Board (IRB), approval numbers TLV-0658-12, TLV-0050-13 and TLV-0522-10; Kfar Shaul Hospital IRB, approval number 0-73; and Weizmann Institute of Science Bioethics and
Embryonic Stem Cell Research oversight committee.
PopCol: approved by the local Committee of Research Ethics (Forskningskommitté Syd) at Karolinska Institutet, Stockholm, in November 2001
RotterdamStudy: approved by the institutional review board (Medical Ethics Committee) of the Erasmus Medical Center and by
the review board of The Netherlands Ministry of Health, Welfare and Sports.
SHIP/SHIP-TREND: approved by medical ethics committee of the University of Greifswald
HCHS/SOL: approved by approval of the Ethics and Institutional Review Boards of all institutions involved (i.e., Bronx Field Center
– Albert Einstein School of Medicine; Chicago Field Center – University of Illinois Chicago; Miami Field Center – University of Miami; San Diego Field Center – San Diego State University)
TwinsUK: approved by the Cornell University IRB (Protocol ID 1108002388)

Note that full information on the approval of the study protocol must also be provided in the manuscript.