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2 **Inter-determination of blood metabolite levels and gut microbiome**
3 **supported by Mendelian randomization**

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26 **Abstract**

27

28 The gut microbiome has been implicated in a variety of physiological states.
29 Controversy over causality, however, has always haunted microbiome studies. Here,
30 we utilized the bidirectional Mendelian randomization (MR) approach to address
31 questions that are not yet mature for more costly randomized interventions. From a
32 total of 3,432 Chinese individuals with shotgun sequencing data for whole genome
33 and whole metagenome, as well as anthropometric and blood metabolic traits, we
34 identified 58 causal relationships between the gut microbiome and blood metabolites,
35 and replicated 43 out of the 58. Gut microbiome could determine features in the blood.
36 For example, increased fecal relative abundances of *Oscillibacter* and *Alistipes* were
37 causally linked to decreased triglyceride concentration, and fecal microbial module
38 pectin degradation might increase serum uric acid. On the other hand, blood features
39 may determine gut microbial features, e.g. glutamic acid appeared to decrease
40 *Oxalobacter*, and a few members of *Proteobacteria* were unidirectionally influenced
41 by cardiometabolically important metabolites such as 5-methyltetrahydrofolic acid,
42 alanine, as well as selenium. This study illustrates the value of human genetic
43 information to help prioritize gut microbial features for mechanistic and clinical studies.
44 The results are consistent with whole-body cross-talks of the microbiome and the
45 circulating molecules.

46

47 **Introduction**

48

49 Metagenome-wide association studies (MWAS) using human stool samples, as well
50 as animal models especially the germ-free mice, have pointed to a potential role of the
51 gut microbiome in diseases such as cardiometabolic, autoimmune, neuropsychiatric
52 diseases and cancer, with mechanistic investigations for diseases such as obesity,
53 colorectal cancer and schizophrenia¹⁻⁴. Twin-based heritability estimation and more
54 recent metagenome-genome-wide association studies (M-GWAS) have questioned
55 the traditional view of the gut microbiota as a purely environmental factor⁵⁻⁹, although
56 the extent of the genetic influence remained controversial^{7,10}. Yet, all these published
57 cohorts, except for human sequences in the metagenomic data of HMP (Human
58 Microbiome Project), utilized array data for human genetics, and most of them had
59 16S rRNA gene amplicon sequencing for the fecal microbiota⁵⁻¹⁰.

60

61 For metabolic traits, a large number of GWAS analyses have been reported¹¹⁻¹⁵. Yet,
62 most of them focused on imputed genotyping array data for the discovery of common
63 variants influencing the human blood metabolome, except for two recent studies^{14,15}
64 which leveraged whole genome or exome sequencing to discover metabolic quanti-
65 tative trait loci (mQTL). These studies consistently indicated high heritability of blood
66 metabolites.

67

68 As the gut microbiome is considered to be highly dynamic, causality has always been
69 an unresolved issue on any reported difference. Mendelian randomization (MR)¹⁶
70 offers an opportunity to distinguish between causal and non-causal effects from
71 cross-sectional data, without animal studies or randomized controlled trials. An early
72 study used MR to look at the gut microbiota and ischemic heart disease¹⁷. Recently, a
73 study used MR to confirm that increased relative abundance of bacteria producing the
74 fecal volatile short-chain fatty acid (SCFA) butyrate was causally linked to improved
75 insulin response to oral glucose challenge; in contrast, another fecal SCFA,
76 propionate, were causally related to an increased risk of T2D¹⁸. However, both studies
77 used genotype data, and it was not clear to what extent the genetic factors explained
78 the microbial feature of interest.

79

80 In this study, we presented the first large-scale M-GWAS using whole genome and
81 fecal microbiome, and bidirectional MR for the fecal microbiome and anthropometric
82 features as well as blood metabolites. In a two-stage design from different cities in
83 China, 58 causal links were identified from MR in the 4D-SZ discovery cohort of 2,002
84 individuals with high-depth whole-genome sequencing data (1,539 individuals with
85 microbiome data for one-sample MR). 43 of the 58 causal effects were replicated in
86 the low-depth whole-genome sequencing data of another 1,430 individuals (1,006
87 individuals with microbiome data for one-sample MR). In general, unidirectional
88 causal effects could be found both from the gut to the blood and from the blood to the
89 gut, but bidirectional effects were rarely detected. A few of the M-GWAS associations
90 with gut microbial functional modules, e.g. module for lactose/galactose degradation

91 and the *ABO* loci, reached study-wise significance, illustrating the power of shotgun
92 metagenomic data together with whole genome. The MR findings were corroborated
93 and extended by summary statistics from the Japan Biobank study, e.g. causal effect
94 of *Proteobacteria* on T2D (Type 2 diabetes mellitus), congestive heart disease and
95 colorectal cancer, underscoring the significance of human genetic data to help guide
96 microbiome intervention studies.

97

98

99 **Results**

100 **Fecal microbiome associated with human genetics**

101 We set out to identify human genetic variants to be included as the randomizing layer
102 of MR (**Fig. 1**). The 4D-SZ (multi-omics, with more time points to come, from
103 Shenzhen, China) discovery cohort consisted of high-depth whole-genome
104 sequencing data from 2,002 blood samples (mean depth of 42x, ranged from 21x to
105 87x, **Supplementary Table 1, Supplementary Fig. 1a**), out of which 1,539
106 individuals had metagenomic shotgun sequencing data from stool samples ($8.56 \pm$
107 2.28 GB, **Supplementary Fig. 1b**). Fecal M-GWAS was performed using 10 million
108 common and low-frequency variants (minor allele frequency (MAF) $\geq 0.5\%$) and 500
109 unique microbial features (120 from the initial 620 microbial taxonomic or functional
110 features was omitted due to strong association with other microbial features,
111 Spearman's correlation > 0.99). The M-GWAS was adjusted for age, gender, BMI,
112 defecation frequency, stool form, self-reported diet, lifestyle factors, and the first four
113 principal components from the genomic data to account for population stratification.

114

115 With this so-far the largest cohort of whole genome and whole metagenome data, we
116 performed M-GWAS analysis and identified a total of 625 associations involving 548
117 independent loci for one or more of the 500 microbial features at genome-wide
118 significance ($P < 5 \times 10^{-8}$). With a more conservative Bonferroni-corrected study-wide
119 significant P value of 1.0×10^{-10} ($= 5 \times 10^{-8} / 500$), we identified 28 associations with
120 fecal microbial features involving 27 genomic loci, of which 5 correlated with gut
121 bacteria and the other 22 associated with gut metabolic pathways (**Supplementary**
122 **Table 2**).

123

124 For MR, it was important for the genetic variants used to be representative of the
125 microbiome features (**Supplementary Fig. 2**), so a more suggestive P value of lower
126 than 1×10^{-5} was used (**Supplementary Table 2**), as in previous MR studies^{18,19}.
127 Each microbial feature had an average of 44 genetic variants (range: 4-262; sd: 38;
128 **Fig. 2a, Supplementary Table 3**). The corresponding genetic variants explained
129 microbial features to a median value of 24.9%, e.g. 45.5% of the microbial metabolic
130 pathway for succinate consumption and 44.6% of *Phascolarctobacterium*
131 *succinatutens* (an asaccharolytic, succinate-utilizing bacterium), while only 6.8% of
132 genus *Edwardsiella* (**Supplementary Table 3**). The phenotypic (relative abundance)
133 variance of five genera *Bilophila*, *Oscillibacter*, *Faecalibacterium*, *Megasphaera* and

134 *Bacteroides* could be explained over 35% by their corresponding independent genetic
135 variants (**Fig. 2b**), and the same is true for species *Bilophila wadsworthia*,
136 *Eubacterium siraeum* and *Faecalibacterium prausnitzii* (a butyrate-producing
137 bacterium that was relatively depleted in metabolic and immune diseases). Thus,
138 although human genetic associations (array data) have been reported to explain only
139 10% or 1.9% of the gut microbiota^{7,10}, the suggestive associations from the current
140 M-GWAS study could be highly predictive of certain gut taxa and functions.

141

142 For better confidence in these suggestive associations, we sequenced a replication
143 cohort of 1,430 individuals from multiple cities in China (also shotgun metagenomic
144 sequencing for stool samples to an average of 8.65 ± 2.42 GB (**Supplementary Fig.**
145 **1d**), but about 8x whole-genome sequencing for human genome, ranged from 6x to
146 16x (**Supplementary Fig. 1c**)). Among the 22,293 independent associations
147 identified in the discovery cohort with $P < 10^{-5}$, 4,876 variants were not available in the
148 low-depth replication dataset and 87.6% of them were not common variants (MAF <
149 0.05), which was understandable given the relatively low detection rate of rare genetic
150 variants from 8x sequencing data. For the remaining 17,417 independent
151 associations covered by the low-depth replication dataset, we were able to replicate
152 2,324 in the same effect direction of minor allele ($P < 0.05$, **Supplementary Table 2**),
153 indicating that the associations were not random false positives. The fraction of
154 associations replicated in the same direction ($P < 0.05$) using the suggestive cut-off of
155 $P < 10^{-5}$ (2,324/17,417) was not lower than the more stringent cut-offs (54/625 of the P
156 $< 5 \times 10^{-8}$, and 2/28 of the $P < 10^{-10}$). Two well replicated signals from the study-wide
157 threshold were chr9:133276163 in the *ABO* blood group associated with module
158 MF0007: lactose and galactose degradation ($P_{discovery} = 2.10 \times 10^{-12}$ and $P_{replication} =$
159 1.09×10^{-10} ; **Supplementary Fig. 3a,b**) and rs142693490 near the *LCORL* gene
160 (implicated in spermatogenesis, body frame and height) associated with MF0034:
161 alanine degradation II ($P_{discovery} = 1.28 \times 10^{-12}$ and $P_{replication} = 0.014$; **Supplementary**
162 **Fig. 3c,d**). Chr9:133276163 is in strong linkage disequilibrium (LD, $r^2 = 0.99$) with
163 multiple SNPs (rs507666, rs532436, rs651007, rs579459 and rs579459) in the *ABO*
164 gene. These SNPs located in a block were found to be associated with metabolites
165 levels in both this study and previous studies, especially for serum alkaline
166 phosphatase levels (**Supplementary Table 4**). Other fecal microbiome associations
167 confirmed by the low-depth genomes included: *AMIGO1* associated with
168 MF0067: glycolysis (preparatory phase); *RAD51B* associated with MF0019: rhamnose
169 degradation; *IPO8* associated with MF0014: arabinose degradation; *LINC00648*
170 associated with *Streptococcus oralis*; *PLEKHF2* associated with MF0050: threonine
171 degradation II; *IPO8* associated with MF0037: leucine degradation; *RTRAF*
172 associated with *Bacteroides xylanisolvens*; *GNB1* associated with *Megasphaera*
173 *elsdenii*; *DOCK8* associated with *Actinomyces* etc. (**Supplementary Table 2**).

174

175 Besides, 175 loci have been previously⁶⁻¹⁰ reported to associate with specific taxa. We
176 were able to replicate 4 of them at nominal significance, including rs147600757
177 associated with Rikenellaceae and rs62273067 associated with *Acidaminococcus*

178 reported by Turpin et al.⁸, rs10115898 associated with *Streptococcus mutans* and
179 rs78859629 associated with *Lactobacillus acidophilus* reported by Rothschild et al.¹⁰.
180 To accommodate the differences in taxonomic resolution between amplicon data and
181 our shotgun data, we obtained a minimal P value for each SNP across all taxa, and
182 replicated 8 of them at the phylum level ($P < 0.05/134 = 3.7 \times 10^{-4}$, with 134 of the 175
183 loci available in this study; **Supplementary Table 5**), especially for rs12354611 and
184 *Bacteroides stercoris* ($P = 8.64 \times 10^{-6}$).

185

186 **Blood metabolic traits associated with human genetics**

187 On the other hand, plasma metabolites are also called on to associate with host
188 genetics (**Fig. 1**). We thus performed whole genome-wide association tests with an
189 additive model on 10 million common and low-frequency variants ($MAF \geq 0.5\%$) for
190 each of the 112 metabolites, with log-transformed relative abundance. We identified a
191 total of 174 associations involving 158 loci that independently associated with one or
192 more of the 112 metabolites at genome-wide significance ($P < 5 \times 10^{-8}$). With a more
193 conservative Bonferroni-corrected study-wide significant P value of 4.5×10^{-10} ($= 5 \times$
194 $10^{-8}/112$ metabolites), we identified 39 associations with metabolites involving 28
195 genomic loci (**Supplementary Table 6**). These included previously well-established
196 associations such as the *UGT1A* family associated with serum total bilirubin^{11,20} and
197 *ASPG* associated with asparaginate¹¹.

198

199 According to the suggestive threshold of $P < 10^{-5}$, we identified 6,541 mQTLs, of
200 which 361 were associated with two or more metabolites. Summary statistics for all
201 independent genetic variants associated with metabolic traits with a P value lower
202 than 1×10^{-5} are included in **Supplementary Table 6**. The average number of
203 genetic variants was 58 for each metabolic trait (range: 14-240; sd: 36, **Fig. 3a**;
204 **Supplementary Table 7**). The percentage of variance explained by the
205 corresponding genetic variants ranged from 13.3% (red blood cell distribution) to as
206 high as 48.3% (blood mercury concentration) and 45.9% (blood alpha-fetoprotein
207 value), with a median value of 28.6% (**Fig. 3b**). Among these, 268 variants or their
208 proxy variants ($r^2 > 0.6$; distance < 1 MB) have been reported in the GWAS catalog²¹
209 (**Supplementary Table 8**). Some variants were associated with diseases in the
210 GWAS catalog such as chronic kidney disease, Alzheimer's disease, coronary artery
211 disease, Crohn's disease, ovarian cancer, breast cancer and gastric cancer.

212

213 Among the 6,541 suggestive mQTLs identified in the 4D-SZ discovery cohort with P
214 $< 10^{-5}$, 5,088 variants were covered by the replication dataset. 717 and 31 were
215 replicated at nominal ($P < 0.05$) and suggestive significance ($P < 10^{-5}$), respectively, in
216 the same effect direction of minor allele (**Supplementary Table 6**). Especially for the
217 174 genome-wide and 39 study-wide significant associations, we could replicate 51
218 and 29 associations in the same direction ($P < 0.05$), respectively. The top
219 associations confirmed by the low-depth genomes ($P < 4.5 \times 10^{-10}$ both in discovery
220 and replication cohorts) included: *FECH* associated with manganese; *UGT1A* family

221 associated with serum total bilirubin as well as direct and indirect (unconjugated)
222 bilirubin; *ASPG* associated with asparagine; *CPS1* associated with glycine; *APOE*
223 associated with low density lipoprotein; *LUC7L* associated with mean corpuscular
224 hemoglobin concentration; *ALAD* associated with lead; *GADL1* associated with
225 beta-alanine; *PRODH* associated with proline; *NPRL3* associated with red blood cell
226 distribution. The association results of the top five traits were shown in
227 **Supplementary Fig. 4**. Overall, the accurate identification of genetic determinants
228 and the high variance explained for both microbial features and blood metabolites are
229 optimal for MR analysis to investigate causality.
230

231 **From observational correlation to Mendelian randomization**

232 As a prerequisite for strong causality, we investigated the correlation between relative
233 abundances of 500 unique fecal microbial features (taxa and functional modules) and
234 112 host metabolic traits using multivariate linear regression. After adjustment for
235 gender and age, we observed 457 significant associations (false discovery rate (FDR)
236 corrected $P < 0.05$, **Supplemental Table 9, online methods**). Three metabolites,
237 glutamic acid, 5-methyltetrahydrofolic acid (5-methyl THF, active form of folic acid)
238 and selenium, were associated with the largest number of microbial features (58, 40
239 and 38, respectively, **Supplementary Fig. 5**). These three metabolites were all
240 associated with the phylum *Proteobacteria* and its constituents, including the family
241 Enterobacteriaceae, genera *Escherichia*, *Methylobacillus* and *Achromobacter*,
242 species *Escherichia coli*, *Pseudomonas stutzeri*, *Achromobacter piechaudii*,
243 *Burkholderia multivorans* and *Methylobacillus flagellates*. Glutamic acid was positively
244 correlated with *Proteobacteria*, whereas 5-methyltetrahydrofolic acid and selenium
245 showed negative correlations with *Proteobacteria*, reminiscent of diverging
246 associations of these metabolites with cardiometabolic diseases and inflammation. In
247 addition to these top three metabolites, *Proteobacteria* also showed the strongest
248 association among gut microbial taxa with another 5 traits (the amino acids
249 hydroxyproline, aspartic acid, cystine, the metal strontium and the hormone
250 aldosterone), suggesting that *Proteobacteria* is an important taxon for this Asian
251 cohort. These associations extend findings from various studies, and suggest
252 quantitative relationships between gut microbial taxa/functions and plasma
253 metabolites.
254

255 To reveal the potential causal effects of the fecal microbial features on blood
256 metabolites or the other way around, we conducted bidirectional Mendelian
257 randomization analysis for the 457 observationally significant associations (FDR
258 corrected $P < 0.05$ between metabolites and microbial features, **Supplemental Table**
259 **9**). For each trait, we selected independent genetic variants associated with the
260 respective features as instruments ($r^2 < 0.1$ and $P < 1 \times 10^{-5}$). Consistent with
261 previous studies^{18,19}, the threshold of $P < 1 \times 10^{-5}$ was used to include more variants
262 and maximize the strength of genetic instruments. This threshold ensured that the
263 genetic instruments were not too weak for the low-depth replication cohort (**Methods**,

264 **Supplementary Fig. 6 and Supplemental Table 10).** The average F statistic, a
265 measure of the strength of these genetic instruments, was 51.4 (standard deviation
266 (SD): 35.8) for the replication cohort, while an F statistic >10 is considered sufficiently
267 informative for MR analyses²². The average microbial variance explained by the
268 genetic instruments was 22.6% for the discovery cohort and 5.09% for the replication
269 cohort (**Supplementary Fig. 2**). These exceeded the commonly reported 1.9%-5% in
270 certain phenotypes due to missing heritability²³.

271

272 As we were fortunate to have all the data in the same cohort, we first performed
273 one-sample MR analysis to identify causal relationships for the 457 observational
274 correlations in the discovery cohort consisting of 1,539 individuals with both metabolic
275 and microbiome traits. We found 58 significant causal effects, of which 17 showed
276 causal effects for gut microbial features on blood metabolic traits and the other 41
277 showed causal effects for blood metabolic traits on gut microbial features ($P < 1.09 \times$
278 $10^{-4} = 0.05/457$; **Fig. 4, Supplementary Table 11**). Only 4 of these were bidirectional.
279 By applying one-sample MR analyses to the replication dataset of 1,006 low-depth
280 genomes as well as metabolic and microbiome traits from individuals in different cities,
281 we could replicate 43 of the 58 causal relationships (in the same direction and $P <$
282 0.05 ; **Supplementary Table 11**), indicating that the effects were not random false
283 positives.

284

285 Moreover, we also used six different two-sample MR methods, which are more
286 commonly performed when only summary statistics are available from two different
287 cohorts, to analyze our data both in the discovery cohort (summary data for 2,002
288 samples with metabolic traits and 1,539 samples with microbial features) and the
289 replication cohort (summary data for 1,430 samples with metabolic traits and 1,006
290 samples with microbial features). The one-sample MR and the two-sample MR
291 analyses showed highly consistent results, and the Spearman's correlation for beta
292 coefficients between one-sample and two-sample MR reached 0.767 for the discovery
293 cohort ($P < 2.2 \times 10^{-16}$). The 58 causal associations identified by one-sample MR were
294 also significant in the two-sample MR analyses. An additional 14 causal associations
295 were identified by the two-sample MR analyses (**Supplementary Table 12**), possibly
296 due to the larger cohort size. We also examined the presence of horizontal pleiotropy
297 by using the MR-PRESSO Global test²⁴. Only one causal association (the negative
298 effect of selenium on the abundance of *Methylobacillus flagellates*, $P_{\text{MR-PRESSO Global test}} =$
299 0.01 ; **Supplementary Table 9**) showed pleiotropy, while all the other 71 causal
300 relationships showed no evidence of pleiotropy ($P > 0.05$). Thus, our MR analyses
301 identified robust causal relationships between blood metabolic traits and specific
302 features of the gut microbiome.

303

304 **Effects of the gut microbiome on blood metabolic traits**

305 As some of the MR-identified relationships appeared linked, hierarchical clustering
306 was performed for the 12 microbial features and 8 blood metabolites involved in the

307 17 causal relationships from the gut microbiome to blood metabolites, which formed
308 two clusters. One cluster involved decreasing the plasma levels of triglyceride and
309 alanine by gut microbial taxa or functional modules; and the other involved decreasing
310 the levels of 5-methyltetrahydrofolic acid or progesterone, but increasing serum uric
311 acid or plasma glutamic acid by gut microbial features (**Fig. 4a**). Reassuringly, the
312 species *Mobiluncus curtisii* was clustered next to its corresponding genus *Mobiluncus*,
313 and modules including serine degradation and threonine degradation, sucrose
314 degradation and pectin degradation, were likewise next to one another.

315
316 The most significant causal effect of *Oscillibacter* on decreasing blood triglyceride
317 concentration (**Fig. 5a-c**), and to a lesser extent on lowering body-mass index (BMI)
318 and waist-hip ratio (WHR), whereas the effect with plasma alanine was bidirectional.
319 Using 134 genetic variants to construct a polygenic risk score (PRS) (134 genetic
320 variants and the constructed PRS explained 39.3% and 49.6% of the phenotypic
321 variance, respectively, **Fig. 3b and Supplementary Table 11**) for one-sample MR
322 analysis in the discovery cohort, we estimated that each 1-s.d. increase in the
323 abundance of *Oscillibacter* would generate a 0.261 mmol/L decrement in triglyceride
324 concentration ($P = 2.53 \times 10^{-10}$), a 0.161 kg/m² decrement in BMI ($P = 1.33 \times 10^{-4}$)
325 and 0.126 ratio decrement in WHR ($P = 2.73 \times 10^{-3}$). This causal relationship was
326 robust when four two-sample MR tests were performed ($P_{\text{GCTA-GSMR}} = 4.34 \times 10^{-11}$,
327 $P_{\text{Inverse_variance_weighted}} = 2.45 \times 10^{-15}$, $P_{\text{weighted-median}} = 1.22 \times 10^{-7}$ and $P_{\text{MR-Egger}} = 1.35 \times$
328 10^{-5}) (**Fig. 5c**), and there was no evidence of horizontal pleiotropy ($P_{\text{MR-PRESSOGlobaltest}} =$
329 0.18 ; **Supplementary Table 12**). The reverse MR analysis (testing the effect of
330 genetic predictors of triglyceride on *Oscillibacter* abundance) was significant but did
331 not reach the multiple test corrected significance ($10^{-4} < P < 0.05$). *Oscillibacter* is a
332 Gram-negative Clostridial bacteria, phylogenetically close to *Oscillospira*²⁵ which
333 could produce valerate or butyrate. In addition, higher relative abundance of *Alistipes*
334 was also associated with decreased blood triglyceride concentration ($P = 8.31 \times 10^{-8}$,
335 **Fig. 5d**). At the species level, both *A. shahii* ($P = 1.37 \times 10^{-6}$) and *unclassified*
336 *Alistipes* sp. HGB5 ($P = 3.36 \times 10^{-5}$) showed negative effects on blood triglyceride.
337 The effect of both *Oscillibacter* and *Alistipes* for lowering blood triglyceride
338 concentration were confirmed in the replication cohort ($P = 3.39 \times 10^{-4}$ and $P = 2.88 \times$
339 10^{-4} , respectively; **Supplementary Table 11 and 12**). These findings support the
340 decrease in relative abundances of *Oscillibacter* and *Alistipes* in obese individuals
341 compared to individuals with normal BMI reported in previous studies²⁶⁻²⁸, suggesting
342 that these bacteria as promising supplementation agents for individuals of a suitable
343 genetic background.

344
345 The gut microbiome potential for pectin degradation II (42.6% of the variance
346 explained by GRS) showed a handful of significant MR hits with blood traits (**Fig. 4a**),
347 including positive effects on alanine ($P = 8.57 \times 10^{-5}$) and serum uric acid ($P = 1.34 \times$
348 10^{-6}), whereas negative effects on progesterone ($P = 6.68 \times 10^{-7}$). *Bacteroidetes* and
349 *Fusobacteria* were the only two phyla that positively correlated with the abundance of
350 pectin degradation II (Spearman rank correlation, $\rho = 0.48$ and 0.15 , respectively),

351 which included the two previously reported pectin-degrading species *Bacteroides*
352 *thetaiotaomicron* and *Fusobacterium varium*^{29,30}. In the 4D-SZ cohort, *F. varium*
353 correlated with pectin degradation II (Spearman's correlation, $\rho = 0.12$) and increased
354 the blood alanine ($P = 0.02$) and serum uric acid ($P = 0.04$); *B. thetaiotaomicron*
355 correlated with pectin degradation II (Spearman's correlation, $\rho = 0.21$) but showed no
356 detectable effect on alanine or uric acid ($P > 0.05$; **Supplementary Fig. 7a,b,d**).
357 Instead, *B. dorei*, the bacterial species most strongly correlated with pectin
358 degradation II (Spearman rank correlation, $\rho = 0.32$, **Supplementary Fig. 7c**),
359 positively contributed to alanine ($P = 0.05$) and serum uric acid levels ($P = 3.40 \times 10^{-4}$;
360 **Supplementary Fig. 7d**).
361

362 **Effects of blood metabolites on gut microbial features**

363 For the 41 causal relationships from blood metabolic traits to gut microbial features
364 (one-sample MR, **Supplementary Table 11**), hierarchical clustering revealed two
365 clusters, one mostly involved decreasing abundance of bacteria by plasma alanine or
366 glutamic acid, the other involved decreasing abundance of bacteria by selenium or
367 5-methyltetrahydrofolic acid (**Fig. 4b**). *F. prausnitzii* showed a negative effect on
368 plasma selenium (**Fig. 4a**), while plasma selenium showed negative effects on gut
369 *Proteobacteria* such as Enterobacteriaceae (e.g. *Escherichia coli*, $P = 3.79 \times 10^{-5}$),
370 *Pseudomonas stutzeri* ($P = 1.06 \times 10^{-6}$), and modules such as arginine degradation II
371 ($P = 2.65 \times 10^{-6}$), succinate conversion to propionate ($P = 3.55 \times 10^{-5}$), and anaerobic
372 fatty acid beta oxidation ($P = 9.71 \times 10^{-5}$) (**Fig. 4b**).
373

374 Bacteria from the phylum *Proteobacteria* were negatively affected by not only
375 selenium, but also 5-methyltetrahydrofolic acid (**Fig. 4b**). We directly verified the
376 effect of 5-methyltetrahydrofolic acid on *Escherichia in vitro*. Supplementing
377 5-methyltetrahydrofolic acid in growth media indeed slowed down the growth of a
378 strain of *Escherichia coli* AM17-9 compared to lower concentrations or absence of
379 5-methyltetrahydrofolic acid (**Supplementary Fig. 8**).
380

381 A handful of bacteria were also affected by glutamic acid. The negative influence of
382 glutamic acid (48 variants with suggestive associations and the constructed PRS
383 explained 24.9% and 25.4% of the phenotypic variance, respectively) on the genus
384 *Oxalobacter* ($P = 1.56 \times 10^{-6}$) may help explain the lower prevalence of *Oxalobacter* in
385 developed countries, besides the lower intake of oxalate and antibiotic use³¹. Whether
386 limiting glutamic acid could raise *Oxalobacter* and prevent kidney stones remains to
387 be tested. Glutamic acid negatively affected melibiose degradation (to glucose,
388 galactose, $P = 2.05 \times 10^{-5}$ from two-sample MR), but showed positive effects on
389 alanine degradation I ($P = 5.46 \times 10^{-5}$), anaerobic fatty acid beta-oxidation ($P = 9.36 \times$
390 10^{-5}), and bidirectional positive effect on serine degradation ($P = 6.85 \times 10^{-7}$ for serine
391 degradation to glutamic acid and $P = 9.90 \times 10^{-6}$ for glutamic acid to serine
392 degradation, respectively).
393

394 Causal relationships with the gut microbiome in the context of diseases

395 We further investigated the effects of the 72 significant causal relationships
396 (**Supplementary Table 12**) involving 40 microbial features and 12 metabolic traits on
397 diseases, by performing two-sample MR analysis using gut microbiome GWAS
398 summary data in this 4D-SZ cohort, together with blood quantitative traits and
399 diseases GWAS summary statistics from Japan Biobank³² (**Fig. 1; Supplementary**
400 **Table 13**), given that Japanese people have a genetic architecture similar to Chinese.
401 Only routine blood parameters but no amino acids, hormones and microelements
402 were included in the Japan Biobank study. Thus, only five of the 72 causal
403 associations, involving triglyceride and serum uric acid were available for further
404 investigation in the Japan Biobank data. The relationship between unclassified
405 Lachnospiraceae bacterium 9_1_43BFAA and uric acid was reciprocal in the 4D-SZ
406 cohort and we could replicate the causal effect of uric acid on increased unclassified
407 Lachnospiraceae bacterium 9_1_43BFAA abundance in the Japanese cohort,
408 whereas the reciprocal effect, i.e. potential effect of unclassified Lachnospiraceae
409 bacterium 9_1_43BFAA on uric acid was not replicated, possibly due to lack of
410 variants in the genotyped Japanese cohort (15 instead of 67, **Supplementary Table**
411 **14**). The other three associations were not replicated maybe due to the same reason.
412 For example, genus *Oscillibacter* had 135 variants with $P < 10^{-5}$ in our summary data
413 but only 15 were available in the Japan Biobank summary data.

414
415 MR inference using our gut microbiome M-GWAS summary data and diseases GWAS
416 summary statistics from Japan Biobank found that *Alistipes* that showed negative
417 effects on blood triglyceride in the 4D-SZ cohort, lowered the risks of cerebral
418 aneurysm (**Supplementary Table 15**, $P = 4.61 \times 10^{-4}$) and hepatocellular carcinoma
419 ($P = 0.045$) in the Japan Biobank cohort. According to the genetic associations we
420 identified for *Proteobacteria*, we were able to see in Japan Biobank disease data that
421 *Proteobacteria* increased the risk of T2D (**Fig. 6a**; $P = 7.61 \times 10^{-4}$, two-sample MR),
422 congestive heart failure ($P = 0.003$) and colorectal cancer ($P = 0.047$). This is
423 consistent with MWAS findings mainly for Enterobacteriaceae¹, and suggest that the
424 metabolites identified above (5-methyltetrahydrofolic acid, selenium) might help
425 prevent the diseases. Folic acid is indeed recommended for heart diseases³³. In
426 addition, *Escherichia coli* increased the risk of urolithiasis (**Fig. 6b**; $P = 0.009$) and
427 hepatocellular carcinoma ($P = 0.04$) while decreased the interstitial lung disease risk
428 ($P = 0.007$). Similarly, *Salmonella enterica* increased prostate cancer risk but
429 decreased interstitial lung disease risk. The Pseudomonadales order was the only
430 microbial feature showing a positive effect on pulmonary tuberculosis. The denitrifying
431 bacteria *Achromobacter* increased the risk of atopic dermatitis ($P = 0.005$), gastric
432 cancer ($P = 0.008$), esophageal cancer ($P = 0.027$) and biliary tract cancer ($P = 0.034$).
433 *Bacteroides intestinalis* which was reported to be relatively depleted in patients of
434 atherosclerotic cardiovascular disease³⁴ was found here to increase with potassium,
435 and *B. intestinalis* showed a negative effect on epilepsy. *Streptococcus parasanguinis*
436 had a positive effect on colorectal cancer and posterior wall thickness

437 (echocardiography), consistent with MWAS studies^{1,34,35}. These results illustrated the
438 potential significance of the gut microbiome-blood metabolite relationships in
439 understanding and preventing cardiometabolic diseases and cancer.

440

441

442 **Discussion**

443

444 In summary, we identified abundant genetic loci to associate with microbial features
445 and metabolic traits, and found 58 causal relationships between the gut microbiome
446 and blood metabolites using one-sample bidirectional MR. 43 out of the 58
447 one-sample MR signals could be replicated in a low-depth genome cohort also from
448 China. Two-sample MR replicated the 58 causal relationships in the same direction
449 and identified an additional 14 causal relationships. Two-sample MR using summary
450 statistics from Japan Biobank identified effects of gut microbial features on diseases,
451 suggesting potential applications of microbiome intervention in cardiometabolic,
452 kidney and lung diseases and cancer. While mechanistic investigations using
453 germ-free mice and reference bacteria strains have been popular, our data-driven
454 analyses underscore the clinical relevance of gut microbes that have not been
455 extensively cultured and characterized, e.g. *Oscillibacter* and *Alistipes* for lowering
456 triglyceride concentration and a number of disease risks, which may be particularly
457 relevant for East Asian regions undergoing rapid changes in lifestyle and disease
458 profiles.

459

460 By applying this MR analysis to explore causality, our results laid further support for
461 several previously reported microbiome-metabolites relationships. For example, *B.*
462 *thetaitaomicron* had been reported to inversely correlate with serum glutamate
463 concentration and was lower in obese individuals³⁶. Consistently, we confirmed that

464 species from Bacteroides, including *B. thetaitaomicron*, *B. intestinalis*, *B. helcogenes*

465 and *B. pectinophilus*, reduced plasma glutamic acid concentration ($10^{-4} < P < 0.05$).

466 We also confirmed that *B. thetaitaomicron* could lower plasma Alpha-amino adipic
467 acid level, weight and WHR ($P < 0.05$). Besides, we found that cysteine negatively
468 correlated with abundance of *Escherichia coli*, which is consistent with previous
469 finding that cysteine inhibited the growth of *Escherichia coli*³⁷.

470

471 Although associations between the gut microbiome and blood features such as amino
472 acids and vitamins have been known for some time, our MR analyses could inspire
473 more mechanistic and interventional studies. The unique data available from the
474 4D-SZ cohort allowed appreciation of overlooked features such as selenium.
475 Selenium compounds were deemed essential for human health and development³⁸. It
476 is beneficial to an organism only in small amounts, while high concentrations of
477 selenium become toxic³⁹. We found that higher amount of blood selenium showed
478 negative effects on some members of the gut microbiome (**Fig. 4b**). Although
479 previous studies reported that *Escherichia coli* had evolved for adaptation to

480 selenate⁴⁰⁻⁴³, the MR result that blood selenium negatively impacted the relative
481 abundance of *Escherichia coli* suggested that it may still be sensitive to selenium.
482 Increased selenium level had adverse effects on several other bacteria from
483 Gammaproteobacteria, including *Achromobacter piechaudii*, *Methylobacillus*
484 *flagellatus*, *Pseudomonas stutzeri*, and *Burkholderia multivorans*. *Pseudomonas*
485 *stutzeri* is a nonfluorescent denitrifying and an opportunistic bacterium⁴⁴. *Burkholderia*
486 *multivorans* is a prominent *B. cepacia* complex species causing infection in people
487 with cystic fibrosis⁴⁵. Interestingly, *F. prausnitzii* from the *Firmicutes* phylum showed a
488 negative effect on plasma selenium. Further studies on such indirect relationships
489 between opportunistic pathogens and commensal bacteria would be illuminating, and
490 could help to better protect individuals who have a genetic risk.

491

492 Nitrogen is a limiting resource for many ecosystems. In the modern human gut
493 microbiome without high intake of nitrite, proteins are probably the major source of
494 nitrogen⁴⁶, and the glutamate-glutamine reservoir is a key buffering mechanism for the
495 inflammatory potential of excess amines^{36,47-50}. The increase in *Proteobacteria* and
496 decrease in Oxalobacteraceae observed in these Chinese individuals no more than
497 30 years old on average could potentially explain susceptibility to cardiometabolic and
498 kidney diseases later in life. The bidirectional link between strontium and
499 *Streptococcus parasanguinis* implies an interplay between water source and
500 cardiovascular diseases^{34,51}.

501

502 Metabolism of polysaccharides that cannot be directly digested by the host is an
503 important function of the colonic microbiome. We found degradation of pectin (or
504 sucrose) to negatively affect progesterone level. This is an interesting possibility to
505 provide scientific support for traditional dietary advice for pregnant women to ensure
506 full-term pregnancy. Hyperuricemia and gout is a growing epidemic in East Asia, and
507 soft drinks containing fructose is a strong factor that is no less important than beer
508 and meat⁵². Gut microbial (*Bacteroides*, *Fusobacterium*) pectin degradation module
509 positively contributed to circulating levels of alanine and uric acid. Further studies on
510 the trans-kingdom metabolic flux of carbon and nitrogen would be necessary for
511 personalized management of uric acid and alanine levels.

512

513 For the nascent field of M-GWAS and microbiome MR, there is also a lot of
514 opportunities for methodological development by statistical experts. Low-frequency
515 microbes are common in an individual's gut and could play physiological or
516 pathological roles^{1,53}. Our MR results for gut microbial species were supported by MR
517 for higher taxonomic units such as genus or phylum (**Fig. 4, Supplementary Table**
518 **11**). Yet, the *P* values were sometimes more significant for the larger taxa, suggesting
519 similar functions contributed by other species. Functional redundancy in the
520 microbiome has been discussed ever since the beginning of the microbiome field^{54,55},
521 and here we identified study-wide significant host genetic associations with gut
522 microbial functional modules, and causal effects of other gut microbial functional
523 modules on host levels of circulating metabolites. Distribution of the microbiome

524 taxonomic or functional data constitutes another layer of consideration, in addition to
525 the human allele frequencies. Gathering a more homogenous cohort could enable
526 identification of signals in a relatively small cohort, while corrections for comparing
527 different populations might involve host-microbiome interactions. As the gut
528 microbiome can be influenced by medication⁵⁶, and heritability for most traits is higher
529 in younger individuals⁵⁷, healthy young adults are probably preferable for M-GWAS
530 studies, while microbiome-drug interactions in older individuals could be an important
531 direction for MR studies.

532

533 In short, our data-driven approach underscores the great potential of M-GWAS and
534 MR for a full picture of the microbiome, which can be mechanistically illuminating and
535 are poised to help focus intervention efforts to mitigate inflammation and prevent or
536 alleviate complex diseases.

537

538 **Methods**

539

540 **Study subjects**

541 All the adult Chinese individuals were recruited for a multi-omic study, with some
542 volunteers having samples from as early as 2015, which would constitute the time
543 dimension in '4D'. The discovery cohort was recruited during a physical examination
544 from March to May in 2017 in the city of Shenzhen, including 2,002 individuals with
545 blood samples and of which 1,539 had fecal samples. All these individuals were
546 enlisted for high-depth whole genome and whole metagenomic sequencing. As for
547 replication, blood samples were collected from 1,430 individuals, out of which 1,006
548 had fecal samples. The replication cohort was designed in the same manner but
549 organized at smaller scales in multiple cities (Wuhan, Qingdao, etc.) in China. The
550 protocols for blood and stool collection, as well as the whole genome and
551 metagenomic sequencing were similar to our previous literature^{5,48}. For blood sample,
552 buffy coat was isolated and DNA was extracted using HiPure Blood DNA Mini Kit
553 (Magen, Cat. no. D3111) according to the manufacturer's protocol. Feces were
554 collected with MGIEasy kit and stool DNA was extracted in accordance with the
555 MetaHIT protocol as described previously⁵⁸. The DNA concentrations from blood and
556 stool samples were estimated by Qubit (Invitrogen). 200 ng of input DNA from blood
557 and stool samples were used for library preparation and then processed for
558 paired-end 100bp and single-end 100bp sequencing, respectively, using BGISEQ-500
559 platform⁵⁹.

560 The study was approved by the Institutional Review Boards (IRB) at BGI-Shenzhen,
561 and all participants provided written informed consent at enrolment.

562

563 **High-depth whole genome sequence for discovery cohort**

564 2,002 individuals in discovery cohort were sequenced to a mean of 42x for whole
565 genome. The reads were aligned to the latest reference human genome
566 GRCh38/hg38 with BWA⁶⁰ (version 0.7.15) with default parameters. The reads
567 consisting of base quality <5 or containing adaptor sequences were filtered out. The
568 alignments were indexed in the BAM format using Samtools⁶¹ (version 0.1.18) and
569 PCR duplicates were marked for downstream filtering using Picardtools (version 1.62).
570 The Genome Analysis Toolkit's (GATK⁶², version 3.8) BaseRecalibrator created
571 recalibration tables to screen known SNPs and INDELS in the BAM files from dbSNP
572 (version 150). GATKlite (v2.2.15) was used for subsequent base quality recalibration
573 and removal of read pairs with improperly aligned segments as determined by Stampy.
574 GATK's HaplotypeCaller were used for variant discovery. GVCFs containing SNVs
575 and INDELS from GATK HaplotypeCaller were combined (CombineGVCFs),
576 genotyped (GenotypeGVCFs), variant score recalibrated (VariantRecalibrator) and
577 filtered (ApplyRecalibration). During the GATK VariantRecalibrator process, we took
578 our variants as inputs and used four standard SNP sets to train the model: (1)
579 HapMap3.3 SNPs; (2) dbSNP build 150 SNPs; (3) 1000 Genomes Project SNPs from
580 Omni 2.5 chip; and (4) 1000G phase1 high confidence SNPs. The sensitivity
581 threshold of 99.9% to SNPs and 99% to INDELS were applied for variant selection

582 after optimizing for Transition to Transversion (TiTv) ratios using the GATK
583 ApplyRecalibration command. After applying the recalibration, there were 60,978,451
584 raw variants left, including 55 million SNPs, and 6 million INDELS.

585 We applied a conservative inclusion threshold for variants: (i) mean depth >8x; (ii)
586 Hardy-Weinberg equilibrium (HWE) $P > 10^{-5}$; and (iii) genotype calling rate > 98%. We
587 demanded samples to meet these criteria: (i) mean sequencing depth > 20x; (ii)
588 variant calling rate > 98%; (iii) no population stratification by performing principal
589 components analysis (PCA) analysis implemented in PLINK⁶³ (version 1.07) and (iv)
590 excluding related individuals by calculating pairwise identity by descent (IBD, Pi-hat
591 threshold of 0.1875) in PLINK. Only 10 samples were removed in quality control
592 filtering. After variant and sample quality control, 1,992 individuals with 6.12 million
593 common (MAF $\geq 5\%$) and 3.90 million low-frequency ($0.5\% \leq \text{MAF} < 5\%$) variants
594 from discovery cohort were left for subsequent analyses.

595

596 **Low-depth whole genome sequence for replicate cohort**

597 1,430 individuals in replication cohort were sequenced to a mean of 8x for whole
598 genome. We used BWA to align the whole genome reads to GRCh38/hg38 and used
599 GATK to perform variants calling by applying the same pipelines as for the high-depth
600 WGS data. After completing the joint calling process with CombineGVCFs and
601 GenotypeGVCFs options, we obtained 43,402,368 raw variants. A more stringent
602 process in the GATK VariantRecalibrator stage compared with the high-depth WGS
603 was then used, the sensitivity threshold of 98.0% to both SNPs and INDELS was
604 applied for variant selection after optimizing for Transition to Transversion (TiTv) ratios
605 using the GATK ApplyRecalibration command. Further, we kept variants with less
606 than 10% missing genotype frequency and minor allele count more than 5. All these
607 high-quality variants were then imputed using BEAGLE 5⁶⁴ with the 1,992 high-depth
608 WGS dataset as reference panel. We retained only variants with imputation info. > 0.7
609 and obtained 10,905,418 imputed variants. We further filtered this dataset to keep
610 variants with Hardy-Weinberg equilibrium $P > 10^{-5}$ and genotype calling rate > 90%.
611 Similar to what we have done for discovery cohort, samples were demanded to have
612 mean sequencing depth > 6x, variant call rate > 98%, no population stratification and
613 no kinship. Finally, 1,430 individuals with 5,884,439 high-quality common and
614 low-frequency variants (MAF $\geq 0.5\%$) from replication cohort were left for subsequent
615 analysis.

616 To assess the data quality, we sequenced 27 samples with both high-depth and
617 low-depth WGS data and then compared the 5,318,809 variants between them for
618 each individual. The average genotype concordance was 98.66% (**Supplementary**
619 **Table 16**).

620

621 **Metagenomic sequencing and profiling**

622 High-quality whole metagenomic sequencing was performed for 1,539 samples from
623 discovery cohort and 1,004 samples from replication cohort with fecal samples
624 available. The reads were aligned to hg38 using SOAP2⁶⁵ (version 2.22; identity ≥ 0.9)
625 to remove human reads. The gene profiles were generated by aligning high-quality

626 sequencing reads to the integrated gene catalog (IGC) by using SOAP2 (identity \geq
627 0.95) as previously described⁵³. The relative abundance profiles of phylum, order,
628 family, class, genera and species were determined from the gene abundances. To
629 eliminate the influence of sequencing depth in comparative analyses, we downsized
630 the unique IGC mapped reads to 20 million for each sample. The relative abundance
631 profiles of gene, phylum, order, family, class, genus and species were determined
632 accordingly using the downsized mapped reads per sample.
633 GMMs (gut metabolic modules) reflect bacterial and archaeal metabolism specific to
634 the human gut, with a focus on anaerobic fermentation processes⁶⁶. The current set of
635 GMMs was built through an extensive review of the literature and metabolic
636 databases, inclusive of MetaCyc⁶⁷ and KEGG, followed by expert curation and
637 delineation of modules and alternative pathways. Finally, we identified 620 common
638 microbial taxa and GMMs present in 50% or more of the samples.

639

640 **Metabolic traits profiling**

641 Measurements of metabolic traits (anthropometric characteristics and blood
642 metabolites) were performed for all the 3,432 individuals during the physical
643 examination in this study. The clinical tests, including blood tests and urinalysis, were
644 performed in licensed physical examination organization. The anthropometric
645 measurements such as height, weight, waistline and hipline were measured by
646 nurses. Age and gender were self-reported. The metabolites, i.e. vitamins, hormones,
647 amino acids and trace elements including heavy metals, were chosen from a health
648 management perspective. Measurements of blood metabolites were performed as
649 described in detail by Jie et al³⁹, blood amino acids were measured by ultra high
650 pressure liquid chromatography (UHPLC) coupled to an AB Sciex Qtrap 5500 mass
651 spectrometry (AB Sciex, US) with the electrospray ionization (ESI) source in positive
652 ion mode using 40 μ l plasma; blood hormones were measured by UHPLC coupled to
653 an AB Sciex Qtrap 5500 mass spectrometry (AB Sciex, US) with the atmospheric
654 pressure chemical ionization (APCI) source in positive ion mode using 250 μ l plasma;
655 blood trace elements were measured by an Agilent 7700x ICP-MS (Agilent
656 Technologies, Tokyo, Japan) equipped with an octupole reaction system (ORS)
657 collision/reaction cell technology to minimize spectral interferences using 200 μ l
658 whole blood; Water-soluble vitamins were measured by UPLC coupled to a Waters
659 Xevo TQ-S Triple Quad mass spectrometry (Waters, USA) with the electrospray
660 ionization (ESI) source in positive ion mode using 200 μ l plasma; Fat-soluble vitamins
661 were measured by UPLC coupled to an AB Sciex Qtrap 4500 mass spectrometry (AB
662 Sciex, USA) with the atmospheric pressure chemical ionization (APCI) source in
663 positive ion mode using 250 μ l plasma.

664

665 **Observational correlation of microbial features with metabolic traits**

666 As many microbial features (taxonomies and pathways) are highly correlated, we first
667 performed a number of Spearman correlation tests and kept only one member of pairs
668 of bacteria or GMMs showing >0.99 correlation coefficient. This filtering resulted in a
669 final set of 500 unique features (99 GMMs and 401 gut taxa) that were used for

670 analyses. We correlated these 500 microbial features with 112 measured metabolic
671 traits, including 9 anthropometric measurements (BMI, WHR, etc.) and 103 blood
672 metabolites (amino acids, vitamins, microelements, etc.) in the 3,432 individuals. All
673 metabolic traits and microbial features were transformed using natural logarithmic
674 function to reduce skewness of distributions. For each phenotype, we excluded outlier
675 individuals with more than four standard deviations away from the mean. The
676 metabolite measures were then centered and scaled to mean of 0 and standard
677 deviation of 1.

678 The relationship between metabolic traits and microbial features were evaluated by
679 multivariable linear regression analysis while adjusted for age and gender. After
680 achieving the raw P value, we used the $p.adjust()$ function in R (v3.2.5)) to perform the
681 multiple test correction and calculated adjusted P values with the
682 Benjamini–Hochberg procedure. The results were considered significant when FDR
683 adjusted P value was <0.05 . The correlated microbial features and metabolic traits,
684 raw P and FDR adjusted P values, were included in the **Supplementary Table 9**.

685

686 **Clustering of microbiome-metabolites associations**

687 To assess the association clusters of 58 identified causal relationships involving the
688 effects of 12 microbial features on 8 metabolic traits and the effects of 7 metabolic
689 traits on 33 microbial features, we performed a hierarchical clustering analysis. Beta
690 coefficients of associations between the microbial features and metabolic traits from
691 one-sample MR analysis were used to construct distance matrices. Complete-linkage
692 hierarchical clustering was used to cluster the metabolites and microbiome traits from
693 the distance matrices using the 'hclust' function in R, and the results were visualized
694 as a heatmap.

695

696 **Genome-wide Association analysis for microbial features**

697 We tested the associations between host genetics and gut bacteria using linear or
698 logistic model based on the abundance of gut bacteria. The abundance of bacteria
699 with occurrence rate over 95% in the cohort was transformed by the natural logarithm
700 and the outlier individual who was located away from the mean by more than four
701 standard deviations was removed, so that the abundance of bacteria could be treated
702 as a quantitative trait. Otherwise, we dichotomized bacteria into presence/absence
703 patterns to prevent zero inflation, then the abundance of bacteria could be treated as
704 a dichotomous trait. Next, for 10 million common and low-frequency variants ($0.5\% \leq$
705 $MAF < 5\%$) identified in the discovery cohort and 5.9 million common and
706 low-frequency variants identified in replication cohort, we performed a standard single
707 variant (SNP/INDEL)-based M-GWAS analysis via PLINK using a linear model for
708 quantitative trait or a logistic model for dichotomous trait. Given the effects of diet and
709 lifestyles on microbial features, we included age, gender, BMI, defecation frequency,
710 stool form, 12 diet and lifestyle factors, as well as the top four principal components
711 (PCs) as covariates for M-GWAS analysis in both the discovery and the replication
712 cohort.

713

714 **Genome-wide Association analysis for anthropometric and metabolic traits**

715 For each of the 112 anthropometric and metabolic traits, the log₁₀-transformed of the
716 median-normalized values was used as a quantitative trait. Samples with missing
717 values and values beyond 4 s.d. from the mean were excluded from association
718 analysis. Each of the 10 million common and low-frequency variants identified in the
719 discovery cohort and the 5.9 million common and low-frequency variants identified in
720 the replication cohort was tested independently using a linear model for quantitative
721 trait implemented in PLINK. Age, gender and the top four PCs were included as
722 covariates.

723

724 **Independent predictor and explained phenotypic variance**

725 For each whole-genome wide association result of microbial features and metabolic
726 traits, we first selected genetic variants that showed association at $P < 1 \times 10^{-5}$ and
727 then performed the linkage disequilibrium (LD) estimation with a threshold of LD $r^2 <$
728 0.1 for clumping analysis to get independent genetic predictors. The P-value
729 threshold of 1×10^{-5} was used for selection of genetic predictors associated with
730 microbial features by maximizing the strength of genetic instruments and the amount
731 of the average genetic variance explained by the genetic predictors in an independent
732 sample. For each microbial feature, we got genetic instruments in discovery cohort
733 using different P thresholds, including 5×10^{-8} , 1×10^{-7} , 1×10^{-6} and 1×10^{-5} . We
734 tested the strength of these instruments under different P thresholds by checking
735 whether they predicted corresponding microbial features in an independent sample
736 (**Supplementary Table 10** and **Supplementary Fig. 6**), we observed that the mean
737 value of instrumental F statistics is 3.57 and on average only 0.28% phenotype
738 variance could be explained by instruments on microbial features when using 5×10^{-8}
739 as instrumental cut-off. Therefore, we used a more liberal threshold of $P < 1 \times 10^{-5}$ to
740 select the instruments for microbial features, and the instrumental mean F statistics
741 reached 51.4 (greater than 10) that indicates a strong instrument. The average
742 phenotypic variance explained by instruments on microbial features was 22.6% for
743 the discovery cohort and 5.09% for the replication cohort (**Supplementary Fig. 2**).
744 For consistency, we used the same threshold and procedure for selecting genetic
745 predictors of metabolic traits in both the discovery and the replication cohort. The LD
746 estimation between variants was calculated in 2,002 samples for the discovery cohort
747 and in 1,430 samples for the replication cohort, respectively. For each phenotype, the
748 variance explained by the corresponding independent genetic predictors was
749 estimated using a restricted the maximum likelihood (REML) model as implemented
750 in the GCTA software⁶⁸. We adjusted for age, gender and the top four PCs in the
751 REML analysis.

752

753 **One-sample MR analysis**

754 To investigate the causal effects between microbial features and metabolic traits
755 available from the same cohort, we first performed one-sample bidirectional MR
756 analysis in discovery cohort, which included 1,539 individuals with both metabolites
757 and microbiome traits. We specified a threshold of $P < 1 \times 10^{-5}$ to select SNP

758 instruments and LD $r^2 < 0.1$ threshold for clumping analysis to get independent
759 genetic variants for MR analysis. Then, an unweighted polygenic risk score (PRS)
760 was calculated for each individual using independent genetic variants from GWAS
761 data. Each SNP was recoded as 0, 1 and 2, depending on the number of trait-specific
762 risk increasing alleles carried by an individual. We performed Instrumental variable (IV)
763 analyses employing two-stage least square regression (TSLS) method. In the first
764 stage, for each exposure trait, association between the GRS and observational
765 phenotype value was assessed using linear regression and predicted fitted values
766 based on the instrument were obtained. In the second stage, linear regression was
767 performed with outcome trait and genetically predicted exposure level from the first
768 stage. In both stages, analyses were adjusted for age, gender and top four principal
769 components of population structure. For each trait, TSLS was performed using 'ivreg'
770 command from the AER package in R. We attempted to replicate the causal effects
771 between traits in replication cohort with 1,004 individuals.

772

773 **Two-sample MR analysis**

774 To maximize the sample size in MR analysis and confirmed the causal effect between
775 microbial features and metabolic traits, we also performed two-sample bidirectional
776 MR analysis using six different methods, including genome-wide complex trait
777 analysis-generalized summary Mendelian randomization (GCTA-GSMR) approach⁶⁹
778 and the other five methods implemented in "TwoSampleMR" R package as a robust
779 validation. A consistent effect across the six methods is less likely to be a false
780 positive. If the genetic variants have horizontally pleiotropic effects but are
781 independent of the effects of the genetic variants on the exposure, this is known as
782 balanced pleiotropy. If all the pleiotropic effects are biasing the estimate in the same
783 direction (directional pleiotropy), this will bias the results (with the exception of the
784 MR-Egger method). We used the MR-PRESSO (mendelian randomization pleiotropy
785 residual sum and outlier) Global test to estimate for the presence of directional
786 pleiotropy.

787 We first performed GWAS analysis for every trait and used summary statistics data for
788 MR analysis. Genetic variants with $P < 1 \times 10^{-5}$ and LD $r^2 < 0.1$ were selected as
789 instrumental variables.

790 The six two-sample MR methods were described as following:

791 **GCTA-GSMR.** GSMR tackled pleiotropy using HEIDI test which assumes that most
792 SNPs are not strongly affected by horizontal pleiotropy and attempt to control
793 SNP-heterogeneity by removing SNP-outliers. The p-value default threshold of 0.01
794 was specified for the HEIDI-outlier analysis to remove horizontal pleiotropic SNPs.
795 After pruning for LD by a clump analysis and filtered for horizontal pleiotropy by the
796 HEIDI-outlier analysis, we got the final independent predictors required for the GSMR
797 analysis.

798 **Inverse-variance weighting (IVW).** The simplest way to obtain a MR estimate using
799 multiple SNPs is to perform an inverse variance weighted (IVW) meta-analysis of
800 each Wald ratio^{70,71}, effectively treating each SNP as a valid natural experiment. We
801 used a multiplicative random effects version of the method, which incorporates

802 between instrument heterogeneity in the confidence intervals (allowing each SNP to
803 have different mean effects).

804 **MR-Egger regression.** This method was adapted from the IVW analysis by allowing
805 a non-zero intercept, which allows the nonhorizontal pleiotropic effect across all SNPs
806 to be unbalanced, or directional^{72,73}. Horizontal pleiotropy refers to the effects of the
807 SNPs on the outcome not mediated by the exposure.

808 **Weighted median.** This method allows for consistent causal effect estimation even if
809 the InSIDE assumption is violated, which allows stronger SNPs to contribute more
810 towards the estimate, and can be obtained by weighting the contribution of each SNP
811 by the inverse variance of its association with the outcome⁷⁴.

812 **Mode-based estimate (MBE).** The mode-based estimator clusters the SNPs into
813 groups based on similarity of causal effects, and returns the causal effect estimate
814 based on the cluster that has the largest number of SNPs⁷⁵. This procedure allows for
815 consistent causal effect estimation even if most instruments are invalid. The weighted
816 mode introduces an extra element similar to IVW and the weighted median, weighting
817 each SNP's contribution to the clustering by the inverse variance of its outcome
818 effect. We tested **Simple mode** and **Weighted mode** method in "TwoSampleMR" R
819 packages.

820

821 **In vitro growth of *Escherichia coli* with 5-methyltetrahydrofolic acid** 822 **supplementation**

823 To directly test the interactions between *Escherichia coli* and 5-methyltetrahydrofolic
824 acid, the anaerobic growth of a strain *Escherichia coli* AM17-9 was characterized at
825 different concentrations of 5-methyltetrahydrofolic acid. The *Escherichia coli* AM17-9,
826 isolated from feces of a male, was routinely grown in Luria-Bertani (LB) broth while
827 supplementing 5-methyltetrahydrofolic acid with concentrations of 0, 1 and 2 ng/ml,
828 respectively. The normal concentration of 5-methyltetrahydrofolic acid in human blood
829 ranged from 4.4 ng/ml to 32.8 ng/ml. The growth of *Escherichia coli* AM17-9 was
830 inhibited when supplementing 5-methyltetrahydrofolic acid from 0 to 2 ng/ml. The
831 optical density at 600 nm (OD600) was measured at intervals of two hours using a
832 microplate reader.

833

834 **MR analyses for diseases in Japan Biobank**

835 We downloaded summary statistics data for 42 diseases and 59 blood quantitative
836 traits in 212,453 Japanese individuals³² (<http://jenger.riken.jp/en/result>,
837 **Supplementary Table 13**). More specifically, the 42 diseases encompassed a
838 wide-range of disease categories; 13 neoplastic diseases, five cardiovascular
839 diseases, four allergic diseases, three infectious diseases, two autoimmune diseases,
840 one metabolic disease, and 14 uncategorized diseases. The 59 quantitative traits
841 were comprised of common blood parameters. By combining these data and the gut
842 microbiome GWAS summary data from discovery cohort with high-depth WGS, we
843 performed the two-sample bidirectional MR analysis to investigate the causal effect
844 between the exposure (40 microbial features and 12 metabolic traits that were
845 involved in the 72 significant causal relationships (**Fig. 4**)) and the outcome (42

846 diseases from BioBank Japan), by applying the GSMR method and the other five MR
847 tests as described in the previous paragraph. For consistency, genetic variants with P
848 $< 1 \times 10^{-5}$ and LD $r^2 < 0.1$ were also selected as instrumental variables for phenotypes
849 in the Japan Biobank study.

850

851

852 **DATA AVAILABILITY**

853 All summary statistics such as associations are available as Supplementary files
854 (Supplementary Table 2 and 6). Individual data are protected at CNGBdb
855 (<https://db.cngb.org/search/project/CNP0000794>).

856

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859 the volunteers for their time and for self-collecting the fecal samples using our kit.

860

861 **Author contributions**

862 H.J. and T.Z. conceived and organized this study. J.W. initiated the overall health
863 project. X.X., H.Y. and S.Z. performed the sample collection and questionnaire
864 collection. X.Liu, T.Z., X.T., H.L., X.Q., J.Z., R.W. and Y.H. generated and processed
865 the whole genome data. Y.Z., X.Lin, Z.Z., H.Z., L.T., Q.W., Z.J., and L.X. generated
866 and processed the metagenome data. X.Liu, X.T., H.Z. and L.T. performed the
867 bioinformatic analyses. K.K. joined in the discussion. X.Liu and H.J. wrote the
868 manuscript. All authors contributed to data and texts in this manuscript.

869

870 **Declaration of interests**

871 The authors declare no competing financial interest.

872

873

874 **Reference**

875

876 1 Wang, J. & Jia, H. Metagenome-wide association studies: fine-mining the microbiome.

877 *Nature reviews. Microbiology* **14**, 508-522, doi:10.1038/nrmicro.2016.83 (2016).

878 2 Moschen, A. R. *et al.* Lipocalin 2 Protects from Inflammation and Tumorigenesis

879 Associated with Gut Microbiota Alterations. *Cell host & microbe* **19**, 455-469,

880 doi:10.1016/j.chom.2016.03.007 (2016).

881 3 Long, X. *et al.* Peptostreptococcus anaerobius promotes colorectal carcinogenesis

882 and modulates tumour immunity. *Nature microbiology* **4**, 2319-2330,

- 883 doi:10.1038/s41564-019-0541-3 (2019).
- 884 4 Zhu, F. *et al.* Transplantation of microbiota from drug-free patients with schizophrenia
885 causes schizophrenia-like abnormal behaviors and dysregulated kynurenine
886 metabolism in mice. *Mol Psychiatry*, doi:10.1038/s41380-019-0475-4 (2019).
- 887 5 Liu, X. *et al.* M-GWAS for the gut microbiome in Chinese adults illuminates on
888 complex diseases. *bioRxiv* (2019).
- 889 6 Bonder, M. J. *et al.* The effect of host genetics on the gut microbiome. *Nature genetics*
890 **48**, 1407-1412, doi:10.1038/ng.3663 (2016).
- 891 7 Wang, J. *et al.* Genome-wide association analysis identifies variation in vitamin D
892 receptor and other host factors influencing the gut microbiota. *Nature genetics* **48**,
893 1396-1406, doi:10.1038/ng.3695 (2016).
- 894 8 Turpin, W. *et al.* Association of host genome with intestinal microbial composition in a
895 large healthy cohort. *Nature genetics* **48**, 1413-1417, doi:10.1038/ng.3693 (2016).
- 896 9 Blekhman, R. *et al.* Host genetic variation impacts microbiome composition across
897 human body sites. *Genome biology* **16**, 191, doi:10.1186/s13059-015-0759-1 (2015).
- 898 10 Rothschild, D. *et al.* Environment dominates over host genetics in shaping human gut
899 microbiota. *Nature* **555**, 210-215, doi:10.1038/nature25973 (2018).
- 900 11 Shin, S. Y. *et al.* An atlas of genetic influences on human blood metabolites. *Nature*
901 *genetics* **46**, 543-550, doi:10.1038/ng.2982 (2014).
- 902 12 Draisma, H. H. M. *et al.* Genome-wide association study identifies novel genetic
903 variants contributing to variation in blood metabolite levels. *Nature communications* **6**,
904 7208, doi:10.1038/ncomms8208 (2015).

- 905 13 Kettunen, J. *et al.* Genome-wide study for circulating metabolites identifies 62 loci and
906 reveals novel systemic effects of LPA. *Nature communications* **7**, 11122,
907 doi:10.1038/ncomms11122 (2016).
- 908 14 Long, T. *et al.* Whole-genome sequencing identifies common-to-rare variants
909 associated with human blood metabolites. *Nature genetics* **49**, 568-578,
910 doi:10.1038/ng.3809 (2017).
- 911 15 Yousri, N. A. *et al.* Whole-exome sequencing identifies common and rare variant
912 metabolic QTLs in a Middle Eastern population. *Nature communications* **9**, 333,
913 doi:10.1038/s41467-017-01972-9 (2018).
- 914 16 Burgess, S., Timpson, N. J., Ebrahim, S. & Davey Smith, G. Mendelian randomization:
915 where are we now and where are we going? *Int J Epidemiol* **44**, 379-388,
916 doi:10.1093/ije/dyv108 (2015).
- 917 17 Yang, Q., Lin, S. L., Kwok, M. K., Leung, G. M. & Schooling, C. M. The Roles of 27
918 Genera of Human Gut Microbiota in Ischemic Heart Disease, Type 2 Diabetes Mellitus,
919 and Their Risk Factors: A Mendelian Randomization Study. *Am J Epidemiol* **187**,
920 1916-1922, doi:10.1093/aje/kwy096 (2018).
- 921 18 Sanna, S. *et al.* Causal relationships among the gut microbiome, short-chain fatty
922 acids and metabolic diseases. *Nature genetics* **51**, 600-605,
923 doi:10.1038/s41588-019-0350-x (2019).
- 924 19 Sakaue, S. *et al.* Trans-biobank analysis with 676,000 individuals elucidates the
925 association of polygenic risk scores of complex traits with human lifespan. *Nature*
926 *Medicine*, doi:10.1038/s41591-020-0785-8 (2020).

- 927 20 Cox, A. J. *et al.* Association of SNPs in the UGT1A gene cluster with total bilirubin and
928 mortality in the Diabetes Heart Study. *Atherosclerosis* **229**, 155-160,
929 doi:10.1016/j.atherosclerosis.2013.04.008 (2013).
- 930 21 MacArthur, J. *et al.* The new NHGRI-EBI Catalog of published genome-wide
931 association studies (GWAS Catalog). *Nucleic Acids Res* **45**, D896-D901,
932 doi:10.1093/nar/gkw1133 (2017).
- 933 22 Teumer, A. Common Methods for Performing Mendelian Randomization. *Front*
934 *Cardiovasc Med* **5**, 51, doi:10.3389/fcvm.2018.00051 (2018).
- 935 23 Manolio, T. A. *et al.* Finding the missing heritability of complex diseases. *Nature* **461**,
936 747-753, doi:10.1038/nature08494 (2009).
- 937 24 Verbanck, M., Chen, C. Y., Neale, B. & Do, R. Detection of widespread horizontal
938 pleiotropy in causal relationships inferred from Mendelian randomization between
939 complex traits and diseases. *Nature genetics* **50**, 693-698,
940 doi:10.1038/s41588-018-0099-7 (2018).
- 941 25 Katano, Y. *et al.* Complete genome sequence of *Oscillibacter valericigenes*
942 Sjm18-20(T) (=NBRC 101213(T)). *Stand Genomic Sci* **6**, 406-414,
943 doi:10.4056/sigs.2826118 (2012).
- 944 26 Thingholm, L. B. *et al.* Obese Individuals with and without Type 2 Diabetes Show
945 Different Gut Microbial Functional Capacity and Composition. *Cell host & microbe* **26**,
946 252-264 e210, doi:10.1016/j.chom.2019.07.004 (2019).
- 947 27 Hu, H. J. *et al.* Obesity Alters the Microbial Community Profile in Korean Adolescents.
948 *PLoS One* **10**, e0134333, doi:10.1371/journal.pone.0134333 (2015).

- 949 28 Tims, S. *et al.* Microbiota conservation and BMI signatures in adult monozygotic twins.
950 *ISME J* **7**, 707-717, doi:10.1038/ismej.2012.146 (2013).
- 951 29 Noack, J., Dongowski, G., Hartmann, L. & Blaut, M. The human gut bacteria
952 *Bacteroides thetaiotaomicron* and *Fusobacterium varium* produce putrescine and
953 spermidine in cecum of pectin-fed gnotobiotic rats. *J Nutr* **130**, 1225-1231,
954 doi:10.1093/jn/130.5.1225 (2000).
- 955 30 Luis, A. S. *et al.* Dietary pectic glycans are degraded by coordinated enzyme
956 pathways in human colonic *Bacteroides*. *Nature microbiology* **3**, 210-219,
957 doi:10.1038/s41564-017-0079-1 (2018).
- 958 31 PeBenito, A. *et al.* Comparative prevalence of *Oxalobacter formigenes* in three human
959 populations. *Sci Rep* **9**, 574, doi:10.1038/s41598-018-36670-z (2019).
- 960 32 Ishigaki, K. *et al.* Large scale genome-wide association study in a Japanese
961 population identified 45 novel susceptibility loci for 22 diseases. *bioRxiv*, 795948,
962 doi:10.1101/795948 (2019).
- 963 33 Huo, Y. *et al.* Efficacy of folic acid therapy in primary prevention of stroke among
964 adults with hypertension in China: the CSPPT randomized clinical trial. *JAMA* **313**,
965 1325-1335, doi:10.1001/jama.2015.2274 (2015).
- 966 34 Jie, Z. *et al.* The gut microbiome in atherosclerotic cardiovascular disease. *Nature*
967 *communications* **8**, 845, doi:10.1038/s41467-017-00900-1 (2017).
- 968 35 Yachida, S. *et al.* Metagenomic and metabolomic analyses reveal distinct
969 stage-specific phenotypes of the gut microbiota in colorectal cancer. *Nature Medicine*
970 **25**, 968-976, doi:10.1038/s41591-019-0458-7 (2019).

- 971 36 Liu, R. *et al.* Gut microbiome and serum metabolome alterations in obesity and after
972 weight-loss intervention. *Nat Med* **23**, 859-868, doi:10.1038/nm.4358 (2017).
- 973 37 Harris, C. L. Cysteine and growth inhibition of *Escherichia coli*: threonine deaminase
974 as the target enzyme. *J Bacteriol* **145**, 1031-1035 (1981).
- 975 38 Rayman, M. P. Selenium and human health. *Lancet* **379**, 1256-1268,
976 doi:10.1016/S0140-6736(11)61452-9 (2012).
- 977 39 MacFarquhar, J. K. *et al.* Acute selenium toxicity associated with a dietary supplement.
978 *Arch Intern Med* **170**, 256-261, doi:10.1001/archinternmed.2009.495 (2010).
- 979 40 Shrift, A. & Kelly, E. Adaptation of *Escherichia coli* to selenate. *Nature* **195**, 732-733,
980 doi:10.1038/195732a0 (1962).
- 981 41 Huber, R. E., Segel, I. H. & Criddle, R. S. Growth of *Escherichia coli* on selenate.
982 *Biochim Biophys Acta* **141**, 573-586, doi:10.1016/0304-4165(67)90186-9 (1967).
- 983 42 Guymer, D., Maillard, J. & Sargent, F. A genetic analysis of in vivo selenate reduction
984 by *Salmonella enterica* serovar Typhimurium LT2 and *Escherichia coli* K12. *Arch*
985 *Microbiol* **191**, 519-528, doi:10.1007/s00203-009-0478-7 (2009).
- 986 43 Yee, N. *et al.* Selenate reductase activity in *Escherichia coli* requires *Isc* iron-sulfur
987 cluster biosynthesis genes. *FEMS Microbiol Lett* **361**, 138-143,
988 doi:10.1111/1574-6968.12623 (2014).
- 989 44 Lalucat, J., Bannasar, A., Bosch, R., Garcia-Valdes, E. & Palleroni, N. J. Biology of
990 *Pseudomonas stutzeri*. *Microbiol Mol Biol Rev* **70**, 510-547,
991 doi:10.1128/MMBR.00047-05 (2006).
- 992 45 Lipuma, J. J. The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol*

- 993 *Rev***23**, 299-323, doi:10.1128/CMR.00068-09 (2010).
- 994 46 Reese, A. T. *et al.* Microbial nitrogen limitation in the mammalian large intestine.
995 *Nature microbiology***3**, 1441-1450, doi:10.1038/s41564-018-0267-7 (2018).
- 996 47 Petrus, P. *et al.* Glutamine Links Obesity to Inflammation in Human White Adipose
997 Tissue. *Cell Metab*, doi:10.1016/j.cmet.2019.11.019 (2019).
- 998 48 Jie, Z. *et al.* A multi-omic cohort as a reference point for promoting a healthy human
999 gut microbiome. *bioRxiv*, 585893, doi:10.1101/585893 (2019).
- 1000 49 Choi, W. M. *et al.* Glutamate Signaling in Hepatic Stellate Cells Drives Alcoholic
1001 Steatosis. *Cell Metab* **30**, 877-889 e877, doi:10.1016/j.cmet.2019.08.001 (2019).
- 1002 50 Kang, D. J. *et al.* Gut microbiota drive the development of neuroinflammatory
1003 response in cirrhosis in mice. *Hepatology* **64**, 1232-1248, doi:10.1002/hep.28696
1004 (2016).
- 1005 51 Long, T. *et al.* Plasma metals and cardiovascular disease in patients with type 2
1006 diabetes. *Environ Int* **129**, 497-506, doi:10.1016/j.envint.2019.05.038 (2019).
- 1007 52 Kuo, C. F., Grainge, M. J., Zhang, W. & Doherty, M. Global epidemiology of gout:
1008 prevalence, incidence and risk factors. *Nat Rev Rheumatol* **11**, 649-662,
1009 doi:10.1038/nrrheum.2015.91 (2015).
- 1010 53 Li, J. *et al.* An integrated catalog of reference genes in the human gut microbiome. *Nat*
1011 *Biotechnol* **32**, 834-841, doi:10.1038/nbt.2942 (2014).
- 1012 54 Qin, J. *et al.* A human gut microbial gene catalogue established by metagenomic
1013 sequencing. *Nature* **464**, 59-65, doi:10.1038/nature08821 (2010).
- 1014 55 Human Microbiome Project, C. Structure, function and diversity of the healthy human

- 1015 microbiome. *Nature* **486**, 207-214, doi:10.1038/nature11234 (2012).
- 1016 56 Maier, L. *et al.* Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature*
1017 **555**, 623-628, doi:10.1038/nature25979 (2018).
- 1018 57 Polderman, T. J. *et al.* Meta-analysis of the heritability of human traits based on fifty
1019 years of twin studies. *Nature genetics* **47**, 702-709, doi:10.1038/ng.3285 (2015).
- 1020 58 Qin, J. *et al.* A metagenome-wide association study of gut microbiota in type 2
1021 diabetes. *Nature* **490**, 55-60, doi:10.1038/nature11450 (2012).
- 1022 59 Fang, C. *et al.* Assessment of the cPAS-based BGISEQ-500 platform for
1023 metagenomic sequencing. *GigaScience* **7**, 1-8, doi:10.1093/gigascience/gix133
1024 (2018).
- 1025 60 Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler
1026 transform. *Bioinformatics* **25**, 1754-1760, doi:10.1093/bioinformatics/btp324 (2009).
- 1027 61 Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**,
1028 2078-2079, doi:10.1093/bioinformatics/btp352 (2009).
- 1029 62 McKenna, A. *et al.* The Genome Analysis Toolkit: a MapReduce framework for
1030 analyzing next-generation DNA sequencing data. *Genome Res* **20**, 1297-1303,
1031 doi:10.1101/gr.107524.110 (2010).
- 1032 63 Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based
1033 linkage analyses. *Am J Hum Genet* **81**, 559-575, doi:10.1086/519795 (2007).
- 1034 64 Browning, B. L., Zhou, Y. & Browning, S. R. A One-Penny Imputed Genome from
1035 Next-Generation Reference Panels. *Am J Hum Genet* **103**, 338-348,
1036 doi:10.1016/j.ajhg.2018.07.015 (2018).

- 1037 65 Li, R. *et al.* SOAP2: an improved ultrafast tool for short read alignment. *Bioinformatics*
1038 25, 1966-1967, doi:10.1093/bioinformatics/btp336 (2009).
- 1039 66 Vieira-Silva, S. *et al.* Species-function relationships shape ecological properties of the
1040 human gut microbiome. *Nature microbiology* 1, 16088,
1041 doi:10.1038/nmicrobiol.2016.88 (2016).
- 1042 67 Caspi, R. *et al.* The MetaCyc database of metabolic pathways and enzymes and the
1043 BioCyc collection of Pathway/Genome Databases. *Nucleic Acids Res* 42, D459-471,
1044 doi:10.1093/nar/gkt1103 (2014).
- 1045 68 Yang, J., Lee, S. H., Goddard, M. E. & Visscher, P. M. GCTA: a tool for genome-wide
1046 complex trait analysis. *Am J Hum Genet* 88, 76-82, doi:10.1016/j.ajhg.2010.11.011
1047 (2011).
- 1048 69 Zhu, Z. *et al.* Causal associations between risk factors and common diseases inferred
1049 from GWAS summary data. *Nature communications* 9, 224,
1050 doi:10.1038/s41467-017-02317-2 (2018).
- 1051 70 Burgess, S., Butterworth, A. & Thompson, S. G. Mendelian randomization analysis
1052 with multiple genetic variants using summarized data. *Genet Epidemiol* 37, 658-665,
1053 doi:10.1002/gepi.21758 (2013).
- 1054 71 Bowden, J. *et al.* A framework for the investigation of pleiotropy in two-sample
1055 summary data Mendelian randomization. *Stat Med* 36, 1783-1802,
1056 doi:10.1002/sim.7221 (2017).
- 1057 72 Bowden, J., Davey Smith, G. & Burgess, S. Mendelian randomization with invalid
1058 instruments: effect estimation and bias detection through Egger regression. *Int J*

- 1059 *Epidemiol***44**, 512-525, doi:10.1093/ije/dyv080 (2015).
- 1060 73 Bowden, J. *et al.* Assessing the suitability of summary data for two-sample Mendelian
1061 randomization analyses using MR-Egger regression: the role of the I² statistic. *Int J*
1062 *Epidemiol***45**, 1961-1974, doi:10.1093/ije/dyw220 (2016).
- 1063 74 Bowden, J., Davey Smith, G., Haycock, P. C. & Burgess, S. Consistent Estimation in
1064 Mendelian Randomization with Some Invalid Instruments Using a Weighted Median
1065 Estimator. *Genet Epidemiol***40**, 304-314, doi:10.1002/gepi.21965 (2016).
- 1066 75 Hartwig, F. P., Davey Smith, G. & Bowden, J. Robust inference in summary data
1067 Mendelian randomization via the zero modal pleiotropy assumption. *Int J Epidemiol***46**,
1068 1985-1998, doi:10.1093/ije/dyx102 (2017).
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- 1070

1071 **Figure legends**

1072

1073 **Figure 1. The design and workflow of this study.** The schematic representation of
1074 our study highlights, for each step, the research question that we sought to answer,
1075 the analysis workflow, the used data and the generalized result. We first performed
1076 metagenome and metabolome GWAS to detect genetic variants associated with
1077 microbial features and metabolic traits, respectively, both in discovery and replication
1078 cohorts (Step 1). We then performed observational analysis to identify which microbial
1079 feature (taxa, GMM) correlated with metabolic traits in this cohort (Step 2). We used
1080 2,545 samples with information of both microbial features and metabolic traits; We
1081 observed 457 significant associations between 500 unique microbial features and 112
1082 anthropometric and blood metabolic traits at a FDR adjusted $P < 0.05$. We then
1083 estimated causal relationships for the 457 observational associations through
1084 bidirectional MR analysis in discovery cohort (Step 3). One-sample BMR detected 58
1085 causal associations between microbial features and blood metabolites after multiple
1086 test correction ($P < 1.09 \times 10^{-4}$); two-sample BMR detected the same associations
1087 and an additional 14 associations. As a validation, we replicated the discovered
1088 causal relationships by using the same MR analysis in an independent replication
1089 cohort (Step 4). Over half (43) of the 58 causal associations were replicated in the
1090 same direction ($P < 0.05$). Finally, we used two-sample MR analysis to investigate the
1091 effects of the identified 72 causal relationships on diseases from Japan Biobank study
1092 (Step 5).

1093

1094 **Figure 2. Independent genetic variants and their explained variance of microbial**
1095 **features. (a)** The density plot showed the distribution of number of independent
1096 genetic variants for 500 unique microbial features at $P < 10^{-5}$. The X-axis indicates the
1097 number of independent genetic variants for each microbial feature (taxon or GMM).
1098 The Y-axis indicates the number of microbial features under a given number of
1099 independent predictors. **(b)** Variance explained by the corresponding independent
1100 genetic variants for each microbial feature was shown. The polar bar plot indicates
1101 how much the independent genetic variants of each common genus (appeared at
1102 least 50% of samples) explained for their phenotypic variance (relative abundance of
1103 each genus). Genera were classified according to their respective phylum which were
1104 marked with different colors. The h^2 was calculated using REML method in GCTA
1105 tools.

1106

1107 **Figure 3. Independent genetic variants and their explained variance of**
1108 **metabolic traits. (a)** The density plot showed the distribution of number of
1109 independent genetic variants for 112 metabolic traits at $P < 10^{-5}$. The X-axis indicates
1110 the number of independent genetic variants for each metabolic trait. The Y-axis
1111 indicates the number of metabolic traits under a given number of independent
1112 predictors. **(b)** Variance explained by the corresponding independent genetic variants
1113 for each metabolic trait was shown. The polar bar plot indicates how much the
1114 independent genetic variants of each metabolic trait explained for their phenotypic

1115 variance. Each metabolic trait was classified into different catalogs which were
1116 marked with different colors. The h^2 was calculated using REML method in GCTA
1117 tools.

1118

1119 **Figure 4. Identifying 58 causal relationships for the microbial features and**
1120 **metabolic traits. (a)** showed the causal effects of 12 specific microbial features on 8
1121 metabolic traits involved in the 17 causal associations from gut microbiome to blood
1122 metabolites. **(b)** showed the causal effects of 7 blood metabolites on 33 microbial
1123 features involved in 41 causal associations from blood metabolites to gut microbiome.
1124 The cells marked with “***” represented 43 of the 58 associations that identified in
1125 discovery cohort were also replicated in replication cohort, while “**” represented the
1126 other 15 only significant in discovery cohort. The cell was colored according to the
1127 beta coefficients from one-sample MR analysis, with red and blue corresponding to
1128 positive and negative associations, respectively.

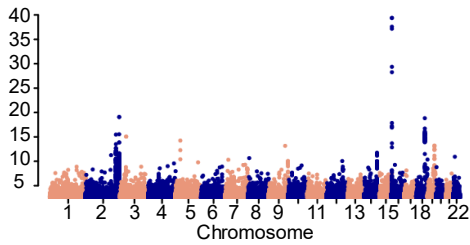
1129

1130 **Figure 5. Causal effects of genus *Oscillibacter* and *Alistipes* on decreasing**
1131 **blood triglyceride concentration. (a)** Schematic representation of the MR analysis
1132 results: genetic predisposition to higher abundance of *Oscillibacter* is associated with
1133 decreased blood triglyceride concentration, to a lesser extent for lowering body mass
1134 index (BMI) and waist-hip ratio (WHR). **(b)** Forest plot represented the effect of per
1135 1-s.d. increase in *Oscillibacter* abundance on blood triglyceride, BMI and WHR, as
1136 estimated using observational and Mendelian randomization (MR) analysis,
1137 respectively. Observational correlation analysis was performed in a total of 2,545
1138 samples (purple). One-sample MR analysis was carried out by using a PGS
1139 constructed by up to 134 genetic predictors as an instrumental variable, as estimated
1140 in discovery cohort (blue) and replication cohort (red), respectively. Corresponding *P*
1141 values from both the observational and MR analysis were shown. CI, confidence
1142 interval. **(c-d)** Forest plots represented the MR estimates and 95% CI values of the
1143 causal effects of *Oscillibacter* **(c)** and *Alistipes* **(d)** on triglyceride level, respectively.
1144 The MR analyses were performed using an one-sample MR and six different
1145 two-sample MR methods both in discovery cohort (blue) and replication cohort (red),
1146 respectively.

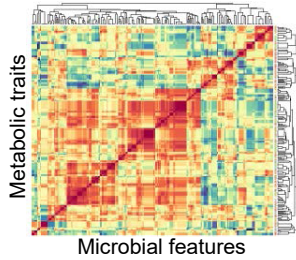
1147

1148 **Figure 6. Causal effects of *Proteobacteria* and *Escherichia coli* on diseases.**
1149 Forest plots represented the MR estimates and 95% CI values of the causal effects of
1150 *Proteobacteria* **(a)** and *Escherichia coli* **(b)** on diseases. The diseases' summary
1151 statistics data was from Japan Biobank study. The gut microbiome GWAS summary
1152 data from this discovery cohort with high-depth WGS was used. Six different
1153 two-sample MR approaches were used. GSMR, generalized summary Mendelian
1154 randomization implemented in GCTA toolbox. IVW, inverse variance weighted. The
1155 corresponding *P* values and β values were shown.

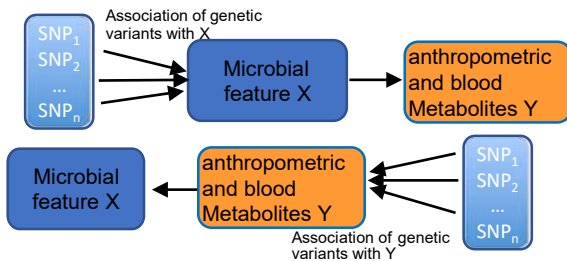
Step 1. What are the genetic variants associated with metabolic traits and microbial features?



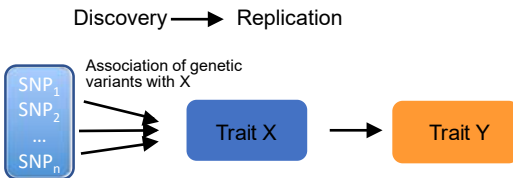
Step 2. Which microbial features correlate with metabolic traits?



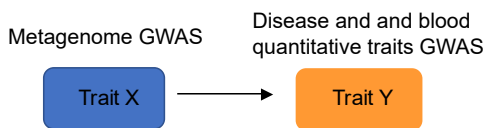
Step 3. Do changes in microbial features causally affect metabolic traits or vice versa?



Step 4. Can we replicate causal relationships?



Step 5. Whether the identified causal relationships have potential link to disease?



Discovery cohort: 2,002 samples with anthropometric and blood metabolites and high-depth whole genome, 1,539 of which with gut metagenome; 10M common and low-frequency variants (MAF > 0.005; HWE $P > 10^{-5}$; variants calling rate > 0.98)

Replication cohort: 1,430 samples with anthropometric and blood metabolites and low-depth whole genome, 1,006 of which with gut metagenome; 5.9M common and low-frequency variants (MAF > 0.005; HWE $P > 10^{-5}$; variants calling rate > 0.98)

Metagenome and metabolome GWAS detected genetic variants associated with microbial features and metabolic traits, respectively.

A total of 3,432 samples with anthropometric and blood metabolic traits and whole genome data, of which 2,545 samples with gut metagenome

Identifying 457 observationally significant associations between 500 unique microbial features and 112 anthropometric and blood metabolites at a FDR adjusted $P < 0.05$ using a multivariable linear regression analysis

One sample BMR detected 58 causal associations between microbial features and blood metabolites after multiple test correction ($P < 1.09 \times 10^{-4}$); two sample BMR detected the same associations and an additional 14 associations

17 causal associations from gut microbiome to blood metabolites involving 12 microbial features and 8 blood metabolites
Two taxa, genus *Oscillibacter* and *Alistipes* decreased triglyceride level.

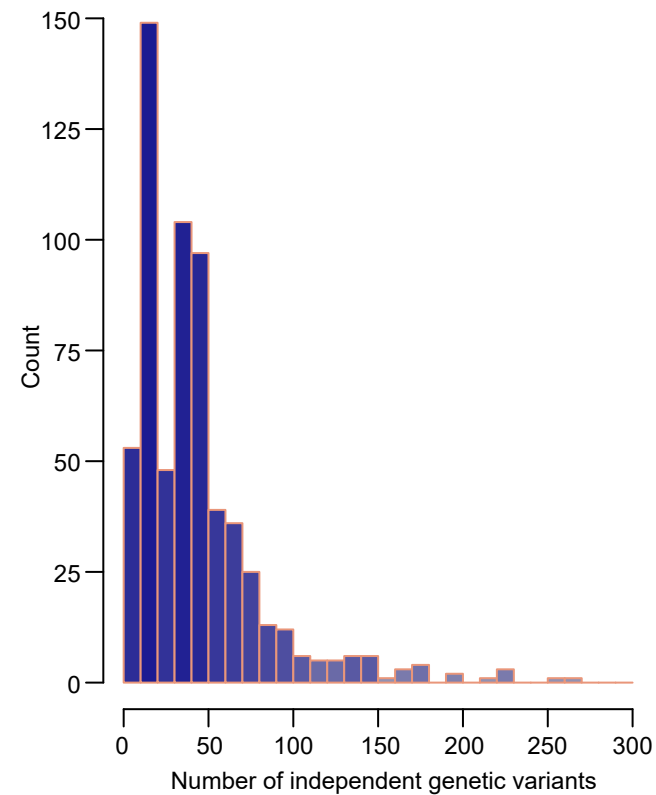
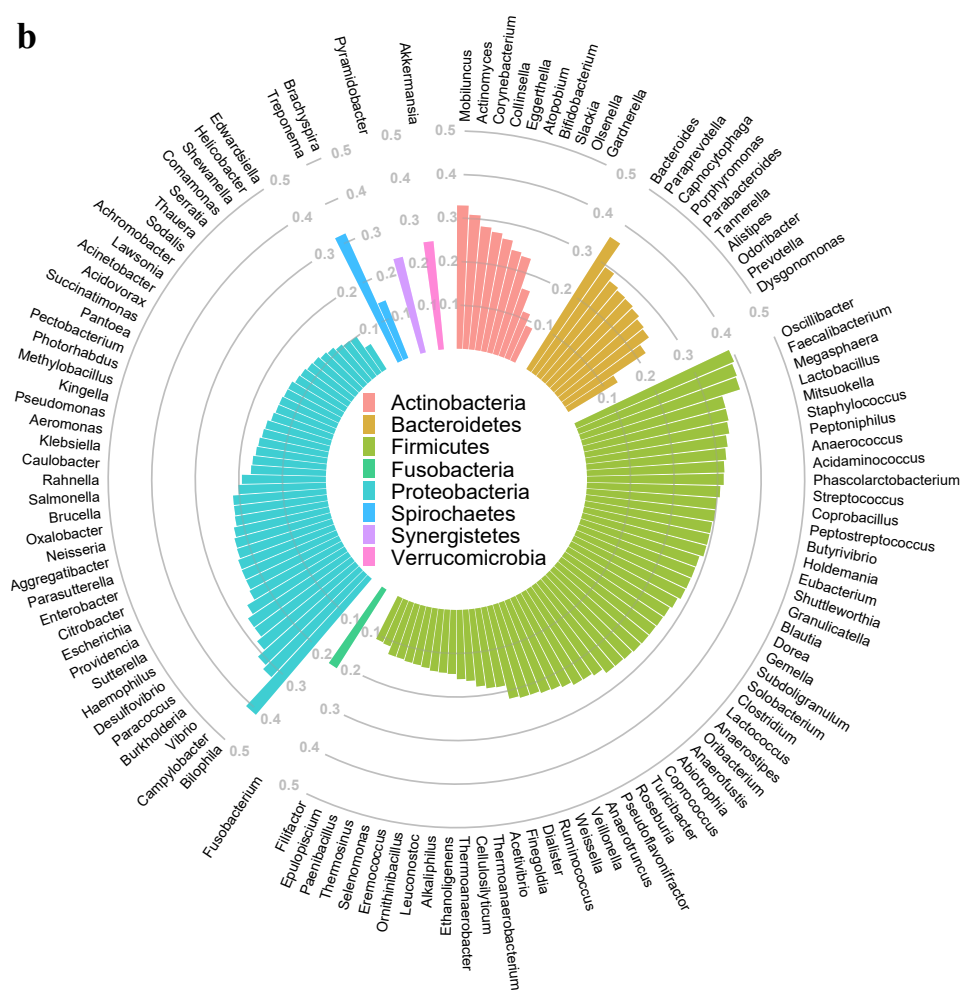
41 causal associations from blood metabolites to gut microbiome involving 7 blood metabolites and 33 microbial features

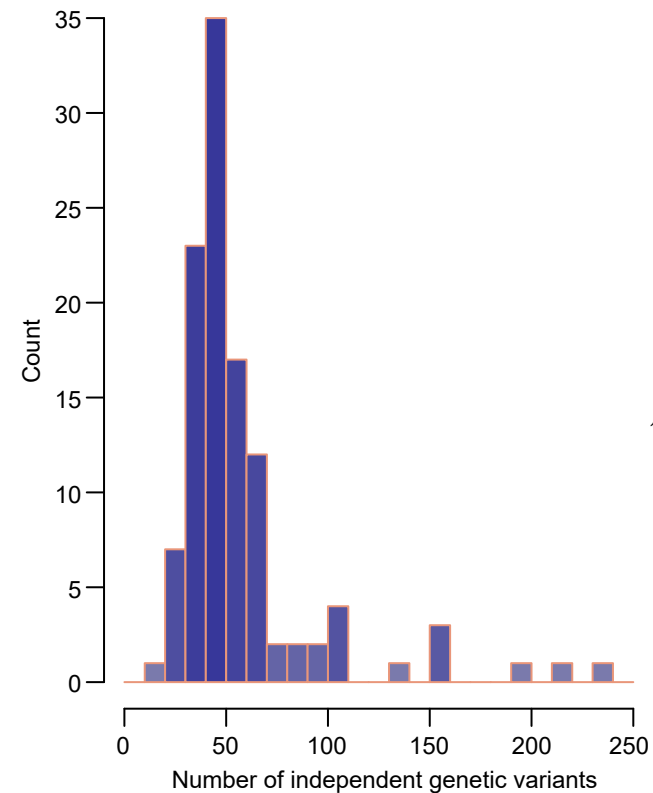
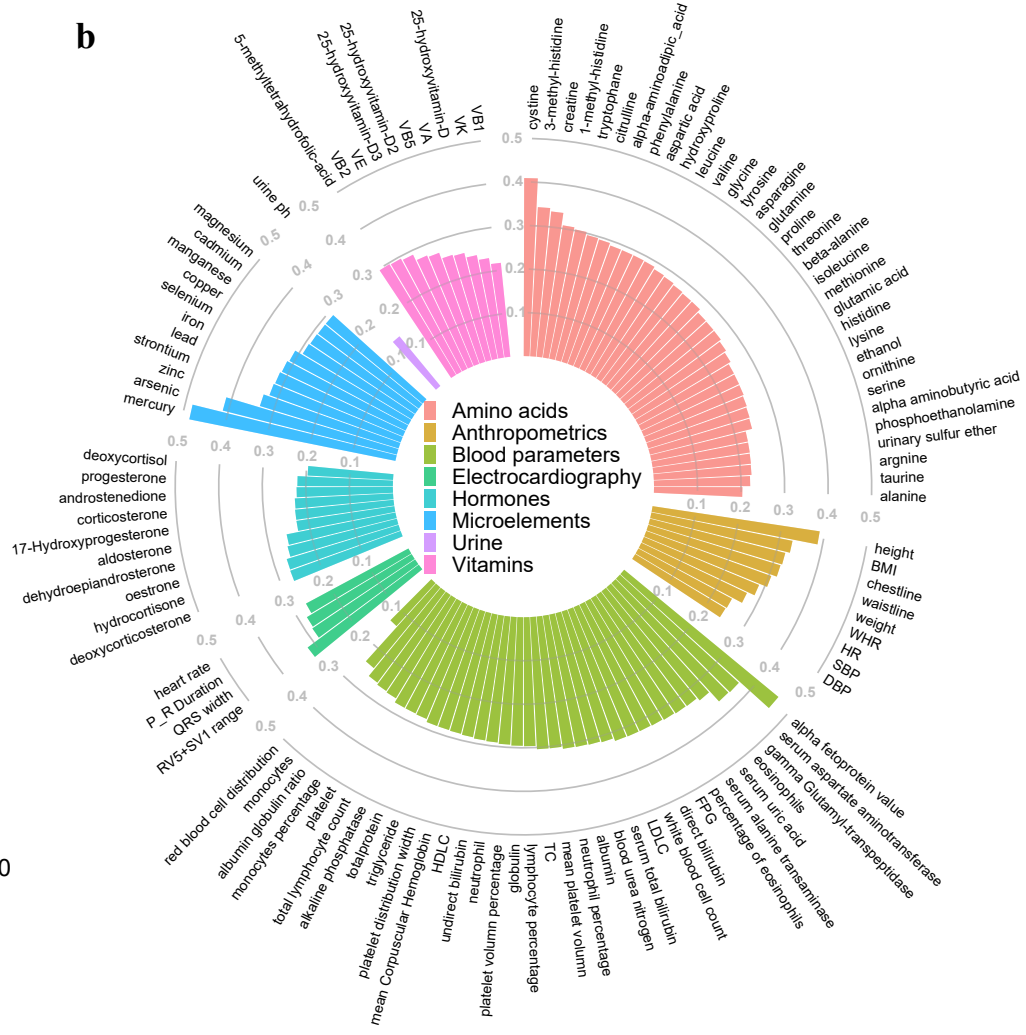
43 of the 58 causal associations were replicated in the same directions and $P < 0.05$ in an independent sample of replication cohort, for example genus *Oscillibacter* and *Alistipes* consistently decreased triglyceride level.

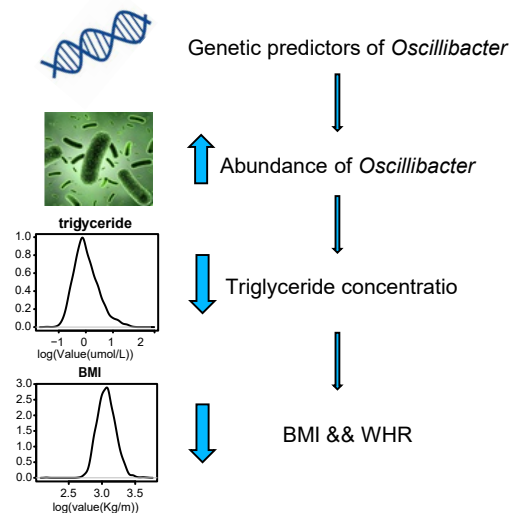
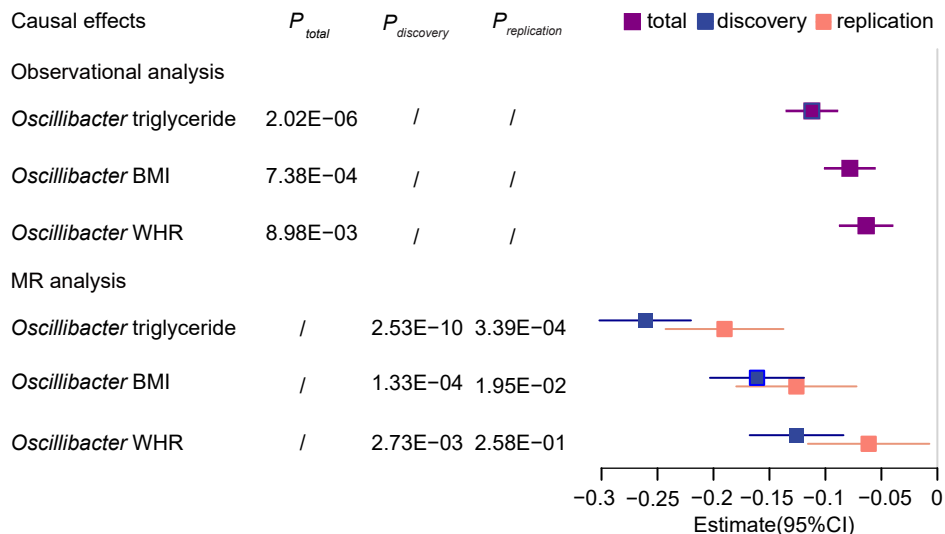
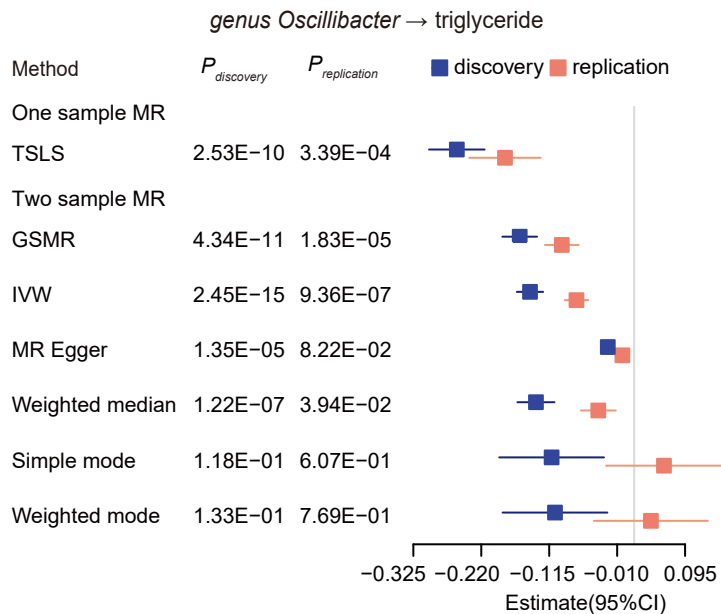
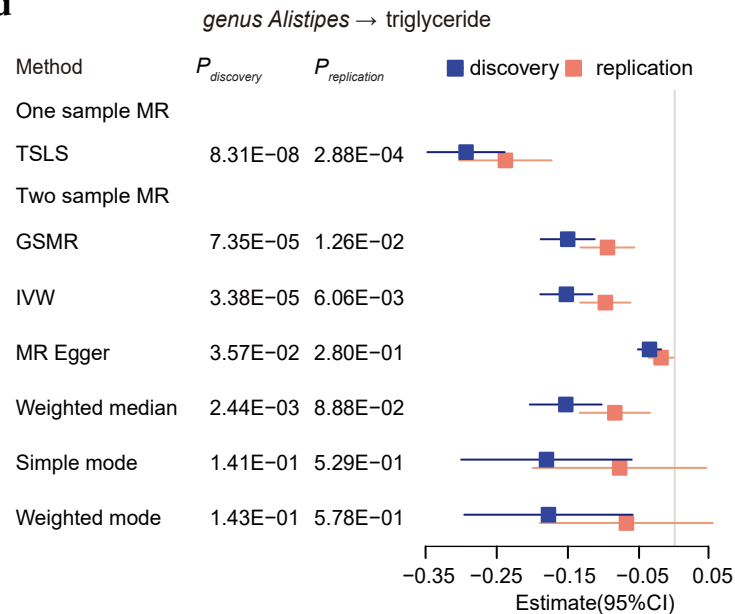
Two sample MR using metagenome GWAS summary data in this study, together with disease and blood quantitative traits GWAS summary data from Biobank Japan study

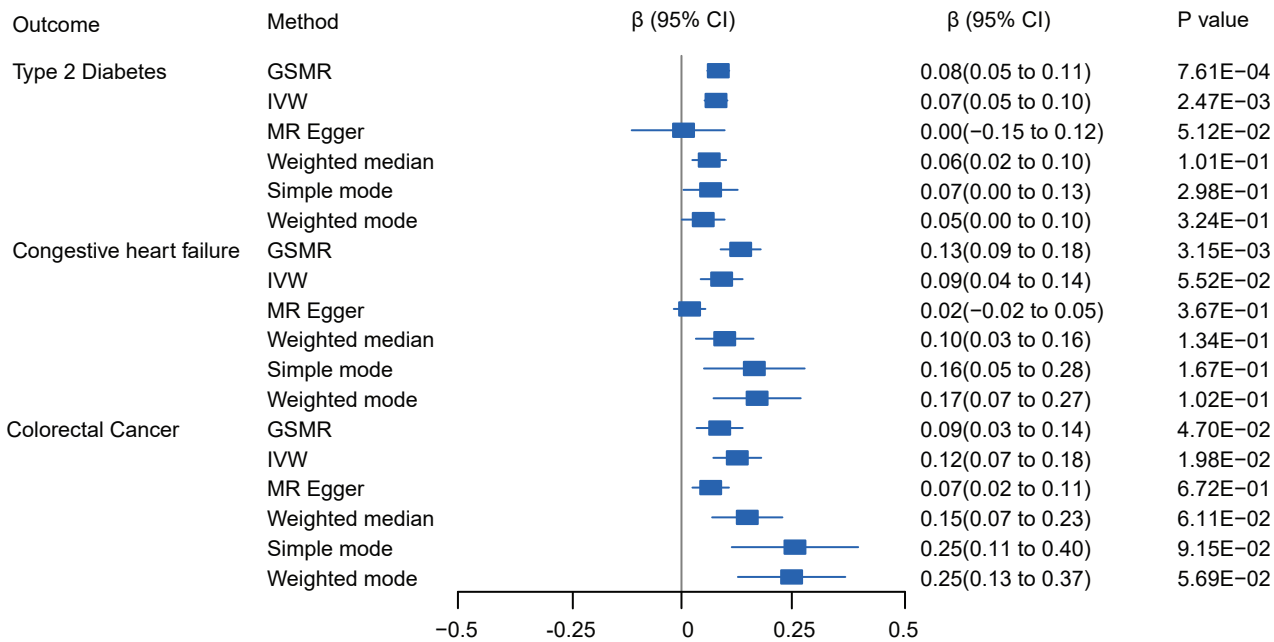
We replicate the causal effect of uric acid on increased species unclassified Lachnospiraceae bacterium 9_1_43BFAA abundance

Some gut bacteria potentially linked to disease risk

a**b**

a**b**

a**b****c****d**

a**b**