The interplay between host genetics and the gut microbiome reveals common and distinct microbiome features for human complex diseases

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Abstract

Background There is increasing interest about the interplay between host genetics and gut microbiome on human complex diseases, with prior evidence mainly derived from animal models. In addition, the shared and distinct microbiome features among human complex diseases remain largely unclear.

Results The analysis was based on a Chinese population with 1,475 participants. We estimated the SNP-based heritability, which suggested that *Desulfovibrionaceae* and *Odoribacter* had significant heritability estimates (0.456 and 0.476, respectively). We performed a microbiome genome-wide association study to identify host genetic variants associated with gut microbiome. We then conducted bi-directional Mendelian randomization analyses to examine the potential causal associations between gut microbiome and human complex diseases. We found that *Saccharibacteria* (per 1-SD higher in the log-transformed abundance) could potentially decrease the concentration of serum creatinine (Beta: -0.011 [95%CI: -0.019, -0.003], p=0.007) and increase estimated glomerular filtration rate (Beta: 0.012 [95%CI: 0.004, 0.020], p=0.003). On the other hand, atrial fibrillation, chronic kidney disease and prostate cancer, as predicted by the host genetics, had potential causal effect on the abundance of some specific gut microbiota. For example, atrial fibrillation (per log odds) could increase the abundance of *Burkholderiales* (Beta: 0.079[95%CI: 0.009, 0.150], p=0.027) and *Alcaligenaceae* (Beta: 0.082[95%CI: 0.012, 0.152], p=0.022), and decrease the abundance of *Lachnobacterium* (Beta:-0.078[95%CI: -0.148, -0.006], p=0.034), *Bacteroides coprophilus* (Beta: -0.113[95%CI: -0.184, -0.041], p=0.002),

Barnesiellaceae (odds ratio: 0.818[95%CI: 0.686, 0.976], p=0.026), *Veillonellaceae* undefined (odds ratio: 0.801[95%CI: 0.669, 0.960], p=0.017) as well as *Mitsuokella* (odds ratio: 0.657[95%CI: 0.496, 0.870], p=0.003). Further disease-microbiome feature analysis suggested that systemic lupus erythematosus and chronic myeloid leukemia shared common gut microbiome features.

Conclusions These results suggest that different human complex diseases share common and distinct gut microbiome features, which may help re-shape our understanding about the disease etiology in humans.

Keywords Gut Microbiome; Host Genetics; Bi-directional Mendelian Randomization Analyses; Disease-Microbiome Features

Background

Ever-increasing evidence has suggested that gut microbiome is involved in many physiological processes, such as energy harvest, immune response, and neurological function [1-3]. With successes of investigation into the clinical application of fecal transplants, modulation of gut microbiome has emerged as a potential treatment option for some complex diseases, including inflammatory bowel disease and colorectal cancer [4, 5]. However, it is still unclear whether the gut microbiome has the potential to be clinically applied for the prevention or treatment of many other complex diseases. Therefore, it is important to clarify the bi-directional causal association between gut microbiome and human complex diseases or traits.

Mendelian randomization (MR) is a method that uses genetic variants as instrumental variables to investigate the causality between an exposure and outcome in observational studies [6]. Prior literatures provide evidence that the composition or structure of the gut microbiome can be influenced by the host genetics [7-10]. On the other hand, host genetic variants associated with gut microbiome are rarely explored in Asian populations, thus we are still lacking instrumental variables to perform MR for gut microbiome in Asians. This calls for novel microbiome genome-wide association study (GWAS) in Asian populations.

Along with the causality issue between the gut microbiome and human complex diseases, it is so far unclear whether human complex diseases had similar or unique

gut microbiome features. Identifying common and distinct gut microbiome features across different diseases may shed light on novel relationships among the complex diseases and update our understanding about the disease etiology in humans. However, the composition and structure of gut microbiome are influenced by a variety of factors including environment, diet and regional variation [11-13], which poses a key challenge for the description of representative microbiome features for a specific disease. Although there were several studies comparing disease-related gut microbiome [14-16], few of them examined and compared the microbiome features across different human complex diseases.

In the present study, we performed a microbiome GWAS in a Chinese cohort study: the Guangzhou Nutrition and Health Study (GNHS) [17], including 1475 participants. Subsequently, we applied a bi-directional MR method to explore the genetically predicted relationship between gut microbiome and human complex diseases. To explore novel relationships among human complex diseases based on gut microbiome, we investigated the shared and distinct gut microbiome features across diverse human complex diseases.

Results

Overview of the study

Our study was based on the GNHS, with 4048 participants (40-75 years old) living in urban Guangzhou city recruited during 2008 and 2013 [17]. In the GNHS, stool

samples were collected among 1937 participants during follow-up visits, among which 1475 unrelated participants without taking anti-biotics were included in our discovery microbiome GWAS. We then included additional 199 participants with both genetic and gut microbiome data as a replication cohort, which belonged to the control arm of a case-control study of hip fracture in Guangdong Province, China [18](See also Figure 1).

SNP-based heritability of gut microbiome

The heritability of alpha diversity ranged from 0.035 to 0.103 (SE: from 0.174 to 0.193, Supplementary Table S3). Significant heritability estimates were observed for several taxa (See also Figure 2, supplementary table S3) with a crude $p<0.05$. To further correct the multiple testing, we calculated the effective number of independent taxa on each taxonomy level(phylum level: 2.3, class level: 2.9, order level: 2.9, family level: 5.5, genus level: 5.6, species level: 3.2), as some taxa were highly correlated with each other. The results suggested that *Desulfovibrionaceae* and *Odoribacter* were heritable ($p<0.05/n$, n is the effective number of independent taxa). Notably, among the suggestively heritable taxa in our cohort, *[Paraprevotellaceae], Veillonellaceae, Desulfovibrionaceae, Pasteurellaceae, Odoribacter, Paraprevotella, Veillonella* and *Bifidobacterium* had nominally significant heritability estimates in prior literatures [7, 19-21].

Association of host genetics with gut microbiome features

We generated categorical variable enterotypes (*Prevotella* vs *Bacteroides*) of the participants based on genus-level relative abundance of gut microbiome [22]. Thereafter, we performed GWAS for enterotypes using logistic regression model to explore potential associations between host genetics and enterotypes. However, we did not find any genome-wide significant locus ($p<5\times10^{-8}$).

To examine the association of host genetic variants with alpha diversity, we performed GWAS for four indices (Shannon diversity index, Chao1 diversity indices, observed OTUs index and Phylogenetic diversity), but again no genome-wide significant signal ($p<5\times10^{-8}$) was found. To further investigate if there is host genetic basis underlying alpha diversity, we constructed a polygenic score for each alpha diversity indicator in the replication cohort, using the genetic variants which showed suggestive significance ($p<5\times10^{-5}$) in the discovery GWAS. The polygenic score was not significantly associated with its corresponding alpha diversity index in our replication cohort. Meanwhile, none of the associations with alpha diversity indices reported in the literature could be replicated (Supplementary Table S8) [7].

The beta diversity GWAS was performed with MicrobiomeGWAS based on Bray–Curtis dissimilarity [23]. We found that one locus at *SMARCA2* gene (rs6475456) was associated with beta-diversity at a genome-wide significance level ($p=3.96\times10^{-9}$). However, we could not replicate the results in the replication cohort, which might be due to the limited sample size of the replication cohort. In addition,

prior literature had reported 73 genetic variants that were associated with beta diversity [8, 13, 24, 25], among which we found that 3 single nucleotide polymorphisms (SNP, *UHRF2* gene-rs563779, *LHFPL3* gene-rs12705241, *CTD-2135J3.4*-rs11986935) had nominal significant ($p<0.05$) association with beta-diversity in our cohort (Supplementary Table S7), although none of the association survived Bonferroni correction. These studies used various methods for the sequencing and calculation of beta diversity, which raised challenges to verify and extrapolate results across populations.

We subsequently performed GWAS discovery for individual gut microbes in our own GNHS discovery dataset. For the taxa (n=114) present in not fewer than ninety percent of participants, we carried out analysis based on a log-normal model. For other taxa (n=88) present in fewer than ninety percent, we transformed the absence/presence of the taxon into binary variables and used a logistic model to prevent zero inflation (Supplementary table S1). For all the gut microbiome taxa, the significant threshold was defined as 5×10^{-8} in the discovery stage. We found that 6 taxa were associated with host genetic variants in the discovery cohort ($p<5\times10^{-8}/n$, n is the effective number of independent taxa on each taxonomy level, Supplementary Table S5); however, these associations were not significant ($p>0.05$) in the replication cohort. We then took the genetic loci reported to be associated with individual taxa in prior studies [7, 8, 13, 25] for replication in our GNHS dataset. Although none of the associations of these genetic variants with taxa survived the Bonferroni correction

(p<1×10-4), we found that *STPG2*-rs4699323 had a nominally significant association (p<0.05) with *Clostridiales* (Beta:-0.131[-0.233- -0.029], p=0.012; Supplementary Table S6). We then used a threshold of $p<5\times10^{-5}$ at the GWAS discovery stage to incorporate additional genetic variants which might explain a larger proportion of heritability for taxa, based on which we constructed a polygenic score for each taxon in the replication. We found that the polygenic scores were significantly associated with 5 taxa including *Saccharibacteria* (also known as *TM7* phylum), *Clostridiaceae*, *Comamonadaceae*, *Klebsiella* and *Desulfovibrio d168* in the replication set (p<0.05, Methods, see also Supplementary Figure 1, Supplementary Table S9).

Genetic correlation of gut microbiome and traits

As the associations of microbiome with complex diseases and traits have been widely reported [26], the genetic correlation between gut microbiome and traits of interest was less clear. Therefore, we applied the bivariate GREML analysis to address this question. The traits included BMI, fasting blood sugar (FBS), glycosylated hemoglobin (HbA1c), systolic blood pressure (SBP), diastolic blood pressure (DBP), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and triglyceride (TG), none of which could pass Bonferroni correction. HDL-C was the only trait that had nominal genetic correlation (p<0.05) with gut microbes (specifically, *Desulfovibrionaceae* and *[Prevotella]*, Supplementary Table S4).

Bi-directional assessment of the genetically predicted association between gut microbiome and complex diseases/traits

Using genetic variants-composed polygenic scores as genetic instruments, we performed MR analysis to assess the putative causal effect of microbiome (*Saccharibacteria*, *Clostridiaceae*, *Comamonadaceae*, *Klebsiella* and *Desulfovibrio d168*) on human complex diseases or traits. Inverse variance weighted (IVW) method was used for the MR analysis, while other three methods (Weighted median, MR-Egger and MR-PRESSO) were applied to confirm the robustness of results. The horizontal pleiotropy was assessed using MR-PRESSO Global test and MR-Egger Regression. For the analysis of gut microbiome on complex traits, we downloaded public available GWAS summary statistics of complex traits (n=58) and diseases (type 2 diabetes mellitus (T2DM), atrial fibrillation (AF), colorectal cancer (CRC) and rheumatoid arthritis (RA)) reported by BioBank Japan [27-32]. The result suggested that *Saccharibacteria* (per 1-SD higher in the log-transformed abundance) could potentially decrease the concentration of serum creatinine (-0.011[95%CI: -0.019, -0.003], p=0.007) and increase estimated glomerular filtration rate(eGFR)(0.012[95%CI: 0.004, 0.020], p=0.003, Supplementary Table S10), which might help improve renal function. We did not find evidence of pleiotropic effect: genetic variants associated with *Saccharibacteria* were not associated with any of the above traits (58 complex traits and 4 disease outcomes, $p<0.05/62$). These taxa were not causally associated with other human complex diseases or traits in our MR analyses, which might be due to the limited genetic instruments discovered in our

present study.

We subsequently performed a reserve MR analysis to assess the potential causal effect of human complex diseases on gut microbiome features. For the reserve MR analyses, the diseases of interests included T2DM, AF, coronary artery disease (CAD), chronic kidney disease (CKD), Alzheimer's disease (AD), CRC and prostatic cancer (PCa), and their instrumental variables for the MR analysis were based on previous large-scale GWAS in East Asians [27, 33-38]. The results suggested that AF and CKD were causally associated with gut microbiome (See also Figure 3A, 3B, Supplementary Table S11). Specifically, genetically predicted higher risk of AF (per log odds) was associated with lower abundance of *Lachnobacterium* (Beta:-0.078[95%CI: -0.148, -0.006], p=0.034), *Bacteroides coprophilus* (Beta: -0.113[95%CI: -0.184, -0.041], p=0.002), *Barnesiellaceae* (odds ratio: 0.818[95%CI: 0.686, 0.976], p=0.026), *Veillonellaceae* undefined (odds ratio: 0.801[95%CI: 0.669, 0.960], p=0.017) as well as *Mitsuokella* (odds ratio: 0.657[95%CI: 0.496, 0.870], p=0.003), and higher abundance of *Burkholderiales* (Beta: 0.079[95%CI: 0.009, 0.150], p=0.027) and *Alcaligenaceae* (Beta: 0.082[95%CI: 0.012, 0.152], p=0.022). Additionally, genetically predicted higher risk of CKD could increase *Anaerostipes* (Beta: 0.291[95%CI: 0.057, 0.524], p=0.015) abundance and higher risk of PCa could decrease *[Prevotella]* (odds ratio: -0.758[95%CI: -1.354, -0.162], p=0.013).

Microbiome features of human complex diseases

To further investigate the potential complex diseases that may be correlated with the taxa affected by AF, we applied Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) to predict the disease pathway abundance [39]. We used Spearman's rank-order correlation to test whether the relative abundances of predicted diseases based on PICRUSt were associated with the aforementioned AF-associated taxa (See also Supplementary Figure 2, Supplementary Table S12). The heatmap indicated that cancers and neurodegenerative diseases including Parkinson's disease (PD), AD, amyotrophic lateral sclerosis (ALS) as well as AF were correlated with similar gut microbiome. Although the association among these diseases are highly supported by previous studies [40-42], no study has compared common gut microbiome features across these different diseases.

To compare gut microbiome features across human diseases, we used the predicted disease abundance based on PICRUSt and performed k-medoids clustering. According to optimum average silhouette width [43], we chose optimal number of clusters for further analysis. The plot showed that neurological diseases including ALS and AD belonged to the same cluster, while PD and CRC had much similarity in gut microbiome. The results also suggested that systemic lupus erythematosus (SLE) and chronic myeloid leukemia (CML) shared similar gut microbiome features (See also Figure 4A, 4B). Moreover, we could replicate these clusters in our replication cohort, which suggested that the clustering results were robust (See also Figure 4C).

We further asked whether gut microbiome contributed to the novel clustering. To this end, we repeated the analysis among participants who took antibiotic less than two weeks before stool sample collection, considering that antibiotic treatments were believed to cause microbiome imbalance. We used the Jaccard similarity coefficient to estimate the cluster difference among GNHS cohort, the replication cohort and the antibiotic group. The similarity between GNHS cohort and the replication cohort was higher than that between GNHS cohort and the antibiotic group (Jaccard similarity coefficient: 0.61 versus 0.11). The results indicated a different clustering, which suggested that gut microbiome indeed contributed to the correlations among diseases (See also Figure 4D). To further demonstrate common microbiome features across different diseases, we examined the correlation of the predicted diseases with genus-level taxa. The results showed that human complex diseases shared similar gut microbiome features, as well as distinct features on their own (See also Figure 5, Supplementary Table S13).

To validate whether the disease-related gut microbiome features annotated by KEGG would be associated with the risk of the disease in real-world community-based cohort, we used T2DM as an example, examining the association of predicted T2DM-related microbiome features with T2DM risk in our GNHS cohort. We constructed a microbiome risk score (MRS) based on 16 selected taxa with predicted correlation coefficients with T2DM greater than 0.2. A logistic regression model was used to examine the association between MRS and T2DM risk in the GNHS (n=1886,

with 217 T2DM cases). The results showed that MRS was positively associated with the risk of T2DM (odds ratio: 1.176[95%CI: 1.114, 1.244], p=8.75×10^{-9}).

Discussion

Our study is among the first to investigate the host genetics-gut microbiome associations in East Asian populations and reveals that several microbiome species (e.g., *Saccharibacteria* and *Klebsiella*) are influenced by host genetics. We found that *Saccharibacteria* might causally improve renal function by affecting renal function biomarkers (i.e., creatinine and eGFR). On the other hand, complex diseases such as atrial fibrillation, chronic kidney disease and prostate cancer, had potential causal effect on gut microbiome. More interestingly, our results indicated that different human complex diseases may be mechanically correlated by sharing common gut microbiome features, but also maintaining their own distinct microbiome features.

Previous studies and our study showed that gut microbiome had an inclination to be influenced by host genetics [8, 10, 25, 44, 45]. The results suggested that *Desulfovibrionaceae* and *Odoribacter* had nominally significant heritability estimates, which were consistent with prior results [7]. We also identified several suggestively heritable taxa, which were found nominally significant in previous literatures [19-21]. In addition, we successfully constructed the polygenic scores for Clostridiaceae and Comamonadaceae, both of which have been identified to be heritable or suggestively heritable [7, 45].

We could not replicate any of the reported genetic variants that were significantly associated with gut microbiome features in prior reports, which might be due to multiple reasons. One of the major reasons may be that the massive multiple testing in insufficiently large samples in prior microbiome GWAS may potentially lead to false positive findings. In addition, other factors including ethnic differences, heterogeneity between studies, gene-environment interaction and dissimilarity in sequencing methods might also make it difficult to extrapolate results from microbiome GWAS across populations in the microbiome field. Nevertheless, we successfully replicated several polygenic scores of gut microbiome. The current study represents the largest dataset, to the best of our knowledge, in Asian populations and may serve as a unique resource for large-scale trans-ethnic meta-analysis of microbiome GWAS in future.

The MR analysis showed that *Saccharibacteria* might decrease the concentration of serum creatinine and increase eGFR. Little is known about the *Saccharibacteria* as one of the uncultivated phyla, and previous studies have shown that it might be essential for the immune response, oral inflammation and inflammatory bowel disease [46-48]. Our results also provided genetic instrument of *Saccharibacteria* for further causal analysis with other complex diseases. The reserve MR analysis provided evidence that AF, CKD and PCa could causally influence gut microbiome. The rare and low-frequency variants may have an important impact on common diseases [49], thus it will be of interest to clarify the effects of low-frequency variants on gut

337 microbiome in cohorts with large sample sizes in future.

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339 Our results indicate that gut microbiome help reveal novel and interesting
340 relationships among human complex diseases, and different diseases may have
341 common and distinct gut microbiome features. A prior study including participants
342 from different countries has identified three microbiome clusters [22]. Notably, this
343 study focused on classifying the individuals into distinct enterotypes regardless of the
344 individuals' health status, while in the present study we described representative
345 microbiome features for diseases of interest. We provided an approach to interpret the
346 data from mechanistic studies based on microbiome. The microbiome features
347 revealed a close association of AF with neurodegenerative diseases as well as cancers,
348 which was supported by prior studies showing that AF had correlation with AD and
349 PD [40, 41], and AF patients had relatively higher risks of several cancers including
350 lung cancer and CRC [42, 50]. We also observed that microbiome features of SLE and
351 CML were highly similar. Interestingly, a tyrosine kinase inhibitor of platelet-derived
352 growth factor receptor, imatinib, was widely used to treat CML and significantly
353 ameliorated survival in murine models of SLE [51]. In addition, close association
354 between CRC and PD has also been reported in several observational cohorts [52, 53].
355 Collectively, these findings strongly supported our hypothesis that human complex
356 diseases sharing similar microbiome features might be mechanically correlated.
357 Furthermore, from the perspectives of risk genes of AF and neurodegenerative
358 diseases, previous GWAS for AF had identified two loci at *PITX2* gene-rs6843082

and *C9orf3* gene-rs7026071, which were also associated with the risk of ALS (p=0.0138 and p=0.049, respectively) [54-56].

We acknowledge several limitations of our study. First, the participants were of East Asian ancestry, and thus factors like ethnic differences and gene-environment interaction might make it difficult to generalize the prior results to our study and extrapolate our results to different ethnic populations. Second, although our analysis included participants with IBD<0.185, the vertical transmission of microbiome from parent to offspring might still partially affect the SNP-based heritability estimates and polygenic scores [20, 57]. Third, genetic factors could explain only a small proportion of variance in gut microbiome features, and thus the power to detect the causal relationship was limited. Therefore large-scale studies are warranted to reveal potential relationships between gut microbiome and complex traits.

Conclusions

In summary, we reveal some causal relationships between abundance of gut microbiome and human complex diseases or traits. The disease and gut microbiome feature analysis reveals novel relationships among human complex diseases, which may help re-shape our understanding about the disease etiology, as well as provide some clues for extending clinical indications of existing drugs for different diseases.

Method

Study participants and sample collection

Our study was based on the Guangzhou Nutrition and Health Study (GNHS), with 4048 participants (40-75 years old) living in urban Guangzhou city recruited during 2008 and 2013 [17]. We followed up participants every three years. In the GNHS, stool samples were collected among 1937 participants during follow-up visits. Among those with stool samples, 1717 participants had genetic data and 1475 participants with identical by decent (IBD) less than 0.185.

We included 199 participants with both genetic and gut microbiome data as a replication cohort, which belonged to the control arm of a case-control study of hip fracture with the participants (52-83 years old) recruited between June 2009 and August 2015 in Guangdong Province, China [18].

Blood samples of all participants were collected after an overnight fasting and buffy coat was separated from whole blood and stored at -80℃. Stool samples were collected during the on-site visit of the participants at Sun Yat-sen University. All samples were manually stirred, separated into tubes and stored at -80℃ within four hours.

Genotyping data

For both discovery and replicattion cohorts, DNA was extracted from leukocyte using the TIANamp® Blood DNA Kit as per the manufacturer's instruction. DNA

concentrations were determined using the Qubit quantification system (Thermo Scientific, Wilmington, DE, US). Extracted DNA was stored at -80°C. Genotyping was carried out with Illumina ASA-750K arrays. Quality control and relatedness filters were performed by PLINK1.9 [58]. Individuals with high or low proportion of heterozygous genotypes (outliers defined as 3 standard deviation) were excluded [59]. Individuals who had different ancestries (the first two principal components ±5 standard deviation from the mean) or related individuals (IBD>0.185) were excluded [59]. Variants were mapping to the 1000 Genomes Phase3 v5 by SHAPEIT [60, 61] and then we conducted the genome-wide genotype imputation with 1000 Genomes Phase3 v5 reference panel by Minimac3 [62, 63]. Genetic variants with imputation accuracy RSQR > 0.3 and MAF > 0.05 were included in our analysis. We used Pan-Asian reference panel consist of 502 participants and SNP2HLA v1.0.3 to impute HLA region [64-66].

Sequencing and processing of 16S rRNA data

Microbial DNA was extracted from fecal samples using the QIAamp® DNA Stool Mini Kit per the manufacturer's instruction. DNA concentrations were determined using the Qubit quantification system. The V3-V4 region of the 16S rRNA gene was amplified from genomic DNA using primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC). The pooled amplicons were sequenced using MiSeq Reagent Kits v2 on the Illuimina MiSeq System with 2 x 250bp pair-end sequencing.

Fastq-files were demultiplexed by the MiSeq Controller Software. Ultra-fast sequence analysis (USEARCH) was performed to trim the sequence for amplification primers, diversity spacers, sequencing adapters, merge-paired and quality filter [67]. The low quality reads (Phred quality scores≤30) were removed. Operational taxonomic units (OTUs) were clustered based on 97% similarity using UPARSE [68]. We removed the OTUs only present in one sample. OTUs were annotated with Greengenes 13_8 (https://greengenes.secondgenome.com/) [69]. After randomly selecting 10000 reads for each sample, Quantitative Insights into Microbial Ecology (QIIME) software version 1.9.0 was used to calculate alpha diversity (Shannon diversity index, Chao1 diversity indices and observed OTUs index and Phylogenetic diversity) based on the rarefied OTU counts [70].

Statistical analysis

Proportion of variance explained by all SNPs

We used the GREML method in GCTA to estimate the proportion of variance explained by all SNPs [71]. The taxa were divided into two groups based on whether the taxa were present in the ninety percent of participants or not. Our model was adjusted for age and sex. The power of GREML analysis was calculated with GCTA power calculator [72].

Genome-wide association analysis of gut microbiome features

For each of the GNHS participants and the replication cohort, we clustered participants based on genus-level relative abundance, estimating the JSD distance and PAM clustering algorithm, and then defined two enterotypes according to Calinski-Harabasz Index [22, 73]. We calculated the genetic principal components of ancestry from genome-wide genetic variants to estimate the population structure. PLINK 1.9 was used to perform a logistic regression model for enterotypes and taxa present in fewer than ninety percent, adjusted for age, sex, sequencing batch and the first five genetic principal components of ancestry.

For beta diversity, the analysis for the genome-wide host genetic variants with beta diversity was performed using MicrobiomeGWAS [23], adjusted for covariates including the first five genetic principal components of ancestry , age and sex.

Alpha diversity was calculated after randomly sampling 10000 reads per sample. For the taxa present in not fewer than ninety percent of participants and alpha diversity, we used Z-score normalization to transform the distribution and carried out analysis based on a log-normal model. A mixed linear model based association (MLMA) test in GCTA was used to assess the association, fitting the first five genetic principal components of ancestry, age, sex and sequencing batch as fixed effects and the effects of all the SNPs as random effects [74-76]. For other taxa present in fewer than ninety percent, we transformed the absence/presence of the taxon into binary variables and used PLINK1.9 to perform a logistic model, adjusted for the first five genetic

principal components of ancestry, age, sex and sequencing batch. For all the gut microbiome features, the significant threshold was defined as 5×10^{-8} /n (n is the effective number of independent taxa on each taxonomy level) in the discovery stage. The QUANTO software was used for power calculations (http://biostats.usc.edu/Quanto.html). We estimated genomic inflation factors with LDSC v1.0.1 at local server [77].

Genetic correlation of gut microbiome and traits

We used GCTA to perform a bivariate GREML analysis to estimate the genetic correlation between gut microbiome and traits in the GNHS [74, 78]. The gut microbiome was divided into two groups according to the previous description. We used continuous variables to taxa present in not fewer than ninety percent of participants. For taxa present in few than ninety percent of participants, we used binary variables according to the absence/presence of taxa. This analysis included traits such as BMI, FBS, HbA1c, SBP, DBP, HDL-C, LDL-C, TC and TG. The power of bivariate GREML analysis was calculated with GCTA power calculator [72].

Constructing polygenic scores for taxa and alpha diversity

We selected lead SNPs using PLINK v1.9 with the '—clump' command to clump SNPs that p value $< 5\times10^{-5}$ and $r^2<0.1$ within 0.1 cM. We used beta coefficients as the weight to construct polygenic scores for taxa and alpha diversity. For alpha diversity

and taxa present in not fewer than ninety percent participants, we constructed weighted polygenic scores and performed the analysis on a general linear model with a negative binomial distribution to test for association between the polygenic scores and taxa, adjusted for the first five genetic principal components of ancestry, age, sex and sequencing batch. We used weighted polygenic scores and logistic regression to the absence/presence taxa, adjusted for the same covariates as in the above analysis. Taxa with significance (p<0.05) in the replication cohort were included for further analysis.

The effective number of independent taxa

As some taxa were correlated with each other, we used an eigendecomposition analysis to calculate the effective number of independent taxa on each taxonomy level [79, 80]. Matrix M is an m×n matrix, where m is the number of participants and n is the number of total taxa on the corresponding taxa level. Matrix A is the variance-covariance matrix of matrix M. P is the matrix of eigenvectors. $\mathrm{diag}\{\lambda_1,\lambda_2,\cdots,\lambda_n\}$ is the diagonal matrix comprised of the ordered eigenvalues, which can be calculated as:

$$\mathrm{diag}\{\lambda_1,\lambda_2,\cdots,\lambda_n\} = P^{-1}AP$$

The effective number of independent taxa can be calculated as:

$$\frac{\left(\sum_{i=1}^{n}\lambda_i\right)^2}{\sum_{i=1}^{n}\lambda_i^2}$$

Bi-directional MR analysis

In the analysis of potential causal effect of gut microbiome features on diseases, we used independent genetic variants (selected as part of the polygenic score analysis) as the instrumental variables. For each trait, we excluded instrumental variables that showed the significant association with the trait ($p<0.05/n$, n is number of independent genetic variants). In the analysis of potential causal effect of diseases on gut microbiome features, we selected genetic variants that were replicated in East Asian populations as instrumental variables. As all instrumental variables were from East Asian populations, we chose independent genetic variants ($r2<0.1$) based on GNHS cohort. We identified the best proxy ($r2 > 0.9$) based on GNHS cohort or discarded the variant if no proxy was available. We used inverse variance weighted (IVW) method to estimate effect size. To confirm the robustness of results, we performed other three MR methods including weighted median, MR-Egger and MR-PRESSO [81-83]. To assess the presence of horizontal pleiotropy, we performed MR-PRESSO Global test and MR-Egger Regression. Effect sizes of gut microbiome on traits were dependent on units of traits (Supplementary table S1). Results of human complex diseases on the absence/presence gut microbiome were presented as risk of presence (vs absence) of the microbiome per log odds difference of the disease. Results of diseases on other gut microbiome were presented as changes in abundance of taxa (1-SD of log transformed) per log odds difference of the respective disease.

The statistical significance of gut microbiome on traits and diseases was defined as $p<0.0008$ (0.05/62). In addition, the statistical significance of diseases on gut

533 microbiome features was defined as $p<0.05/n$ (n is the effective number of
534 independent taxa on the corresponding taxonomy level). Results that could not pass
535 Bonferroni adjustment but $p<0.05$ in all four MR methods were considered as
536 potential causal relationships. We performed MR analyses on R v3.5.3.
537
538 **Pathway analysis**
539 We used OTUs by QIIME and annotated the variation of functional genes with
540 Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
541 (PICRUSt) [39]. The pathways and diseases were annotated using KEGG [84-86]. We
542 used Spearman's rank-order correlation to investigate the association of predicted
543 pathway or diseases abundance with the AF-associated taxa and genus-level taxa. In
544 the heatmap, diseases were clustered with 'hcluster' function on R. To test whether
545 non-normalized pathway or disease abundance was associated with each other, we
546 used SPIEC-EASI to test the interaction relationship, and then used Cytoscape v3.7.2
547 to visualize the interaction network [87, 88].
548
549 **Construction of the microbiome risk score**
550 The microbiome risk score was constructed to validate the accuracy of the association
551 between the predicted disease-related gut microbiome features and the corresponding
552 disease. As we have a large sample size for T2DM cases (n=217 cases) in our cohort,
553 we constructed a microbiome risk score of T2DM as an exemplar. We used a
554 Spearman's rank-order correlation to select taxa with the absolute value of correlation

coefficient higher than 0.2. Score for each taxon abundance <5% quantile in our study was defined as 0. For those above 5%, score for each taxon showing inverse association with T2DM was defined as -1; score for each taxon showing positive association with T2DM was defined as 1. We then summed up values from all taxa. We selected logistic regression model to estimate association of the MRS with T2DM risk, and linear model to estimate the association of the MRS with the continuous variables, adjusted for age, sex, dietary energy intake, alcohol intake and BMI at the time of sample collection.

Clustering diseases

The clustering analysis was carried out with 'cluster' and 'factoextra' for plot on R. We performed PAM algorithm based on predicted abundance of diseases or average relative abundance after Z-score normalization [89]. PAM algorithm searches k medoids among the observations and then found nearest medoids to minimize the dissimilarity among clusters [90]. Given a set of objects $x = (x_1, x_2,...,x_n)$, the dissimilarity between objects x_i and x_j is denoted by $\mathrm{d}(i,j)$. The assignment of object i to the representative object j is denoted by z_{ij}. z_{ij} is a binary variable and is 1 if object i belongs to the cluster of the representative object j. The function to minimize the model is given by:

$$\sum_{i=1}^{n}\sum_{j=1}^{n} d(i,j)z_{ij}$$

To identify the optimal cluster number, we used 'pamk' function in R to determine the optimum average silhouette width. For each object i, we defined N_i as the average

dissimilarity between object i and all other objects within its cluster. For the remaining clusters, $b(i,w)$ represents the average dissimilarity between i and all objects in cluster w. The minimum dissimilarity M_i can be calculated by:

$$M_i = min\forall w(b(i,w)).$$

The silhouette width for object i can be calculated by:

$$sw_i = \frac{M_i - N_i}{max(M_i, N_i)}$$

Then we calculated the average of silhouette width for each object. The cluster number is determined by the number of which the average silhouette width is maximum. We estimated the Jaccard similarity coefficient to quantify the cluster difference between groups. The Jaccard similarity coefficient is positively associated with the similarity of clusters. Given object i and j, as well as group A and B, there are four situations as follows:

(1) S1: in both group A and B, object i and j belong to the same cluster;

(2) S2: in group A, object i and j belong to the same cluster; in group B, they belong to different clusters;

(3) S3: in group A, object i and j belong to different clusters; in group A, they belong to the same cluster;

(4) S4: in both group A and B, object i and j belong to different clusters.

a, b, c and d represents the number of S1, S2, S3 and S4, respectively. The Jaccard similarity coefficient can be calculated by the following formula:

$$J = \frac{a}{a+b+c}$$

Availability of data and materials

The raw data for 16S rRNA gene sequences are available in the CNSA (https://db.cngb.org/cnsa/) of CNGBdb at accession number CNP0000829. Original R scripts are available in GitHub (https://github.com/hsufengzhe/microbiome/tree/master). Request for the metadata from this study can be submitted via email to zhengjusheng@westlake.edu.cn. The proposal is also required for approval.

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Ethics approval and consent to participate

This study was approved by Westlake University Ethics Committee (20190114ZJS003).

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

Authors' contributions

JSZ, YMC and JW initiated and led the study. JY assisted with the data analyses. FZX, YQF and JSZ analyzed the data and wrote the manuscript. TYS and CWL collected the data. ZLJ, ZLM, MLS and WLG analyzed the data. All authors read and approved the final manuscript.

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References

1. Awany D, Allali I, Dalvie S, Hemmings S, Mwaikono KS, Thomford NE, et al. Host and Microbiome Genome-Wide Association Studies: Current State and Challenges. Frontiers in genetics. 2019;9:637-; doi:10.3389/fgene.2018.00637.
2. Bull MJ, Plummer NT. Part 1: The Human Gut Microbiome in Health and Disease. Integrative medicine (Encinitas, Calif). 2014;13(6):17-22.
3. Lynch JB, Hsiao EY. Microbiomes as sources of emergent host phenotypes. Science. 2019;365(6460):1405-9; doi:10.1126/science.aay0240.
4. Allegretti JR, Mullish BH, Kelly C, Fischer M. The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications. The Lancet. 2019;394(10196):420-31; doi:10.1016/S0140-6736(19)31266-8.
5. Wong SH, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. Nature Reviews Gastroenterology & Hepatology. 2019; doi:10.1038/s41575-019-0209-8.
6. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ. 2018;362:k601; doi:10.1136/bmj.k601.
7. Turpin W, Espin-Garcia O, Xu W, Silverberg MS, Kevans D, Smith MI, et al. Association of host genome with intestinal microbial composition in a large healthy cohort. Nat Genet. 2016;48(11):1413-7; doi:10.1038/ng.3693.
8. Wang J, Thingholm LB, Skieceviciene J, Rausch P, Kummen M, Hov JR, et al. Genome-wide association analysis identifies variation in vitamin D receptor and other host factors influencing the gut microbiota. Nat Genet. 2016;48(11):1396-406; doi:10.1038/ng.3695.
9. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, et al. Human genetics shape the gut microbiome. Cell. 2014;159(4):789-99; doi:10.1016/j.cell.2014.09.053.
10. Goodrich JK, Davenport ER, Beaumont M, Jackson MA, Knight R, Ober C, et al. Genetic Determinants of the Gut Microbiome in UK Twins. Cell Host Microbe. 2016;19(5):731-43; doi:10.1016/j.chom.2016.04.017.
11. Ganesan K, Chung SK, Vanamala J, Xu B. Causal Relationship between Diet-Induced Gut Microbiota Changes and Diabetes: A Novel Strategy to Transplant Faecalibacterium prausnitzii in Preventing Diabetes. Int J Mol Sci. 2018;19(12); doi:10.3390/ijms19123720.
12. He Y, Wu W, Zheng HM, Li P, McDonald D, Sheng HF, et al. Regional variation limits applications of healthy gut microbiome reference ranges and disease models. Nat Med. 2018;24(10):1532-5; doi:10.1038/s41591-018-0164-x.
13. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, et al. Environment dominates over host genetics in shaping human gut microbiota. Nature. 2018;555(7695):210-5; doi:10.1038/nature25973.
14. Duvallet C, Gibbons SM, Gurry T, Irizarry RA, Alm EJ. Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. Nature communications. 2017;8(1):1784-; doi:10.1038/s41467-017-01973-8.

15. Cheng S, Han B, Ding M, Wen Y, Ma M, Zhang L, et al. Identifying psychiatric disorder-associated gut microbiota using microbiota-related gene set enrichment analysis. Briefings in Bioinformatics. 2019; doi:10.1093/bib/bbz034.
16. Jackson MA, Verdi S, Maxan M-E, Shin CM, Zierer J, Bowyer RCE, et al. Gut microbiota associations with common diseases and prescription medications in a population-based cohort. Nature Communications. 2018;9(1):2655; doi:10.1038/s41467-018-05184-7.
17. Cao Y, Wang C, Guan K, Xu Y, Su Y-X, Chen YM. Association of magnesium in serum and urine with carotid intima-media thickness and serum lipids in middle-aged and elderly Chinese: a community-based cross-sectional study. European journal of nutrition. 2015;55; doi:10.1007/s00394-015-0839-8.
18. Sun L-L, Li B-L, Xie H-L, Fan F, Yu W-Z, Wu B-H, et al. Associations between the dietary intake of antioxidant nutrients and the risk of hip fracture in elderly Chinese: A case-control study. The British journal of nutrition. 2014;112:1-9; doi:10.1017/S0007114514002773.
19. Lim MY, You HJ, Yoon HS, Kwon B, Lee JY, Lee S, et al. The effect of heritability and host genetics on the gut microbiota and metabolic syndrome. Gut. 2017;66(6):1031-8; doi:10.1136/gutjnl-2015-311326.
20. Davenport ER. Elucidating the role of the host genome in shaping microbiome composition. Gut microbes. 2016;7(2):178-84; doi:10.1080/19490976.2016.1155022.
21. Davenport ER, Cusanovich DA, Michelini K, Barreiro LB, Ober C, Gilad Y. Genome-Wide Association Studies of the Human Gut Microbiota. PloS one. 2015;10(11):e0140301-e; doi:10.1371/journal.pone.0140301.
22. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature. 2011;473(7346):174-80; doi:10.1038/nature09944.
23. Hua X, Song L, Yu G, Goedert JJ, Abnet CC, Landi MT, et al. MicrobiomeGWAS: a tool for identifying host genetic variants associated with microbiome composition. bioRxiv. 2015:031187; doi:10.1101/031187.
24. Ruhlemann MC, Degenhardt F, Thingholm LB, Wang J, Skieceviciene J, Rausch P, et al. Application of the distance-based F test in an mGWAS investigating beta diversity of intestinal microbiota identifies variants in SLC9A8 (NHE8) and 3 other loci. Gut Microbes. 2018;9(1):68-75; doi:10.1080/19490976.2017.1356979.
25. Bonder MJ, Kurilshikov A, Tigchelaar EF, Mujagic Z, Imhann F, Vila AV, et al. The effect of host genetics on the gut microbiome. Nat Genet. 2016;48(11):1407-12; doi:10.1038/ng.3663.
26. Tang WHW, Kitai T, Hazen SL. Gut Microbiota in Cardiovascular Health and Disease. Circulation research. 2017;120(7):1183-96; doi:10.1161/CIRCRESAHA.117.309715.
27. Low SK, Takahashi A, Ebana Y, Ozaki K, Christophersen IE, Ellinor PT, et al. Identification of six new genetic loci associated with atrial fibrillation in the

Japanese population. Nat Genet. 2017;49(6):953-8; doi:10.1038/ng.3842.
28. Suzuki K, Akiyama M, Ishigaki K, Kanai M, Hosoe J, Shojima N, et al. Identification of 28 new susceptibility loci for type 2 diabetes in the Japanese population. Nat Genet. 2019;51(3):379-86; doi:10.1038/s41588-018-0332-4.
29. Akiyama M, Okada Y, Kanai M, Takahashi A, Momozawa Y, Ikeda M, et al. Genome-wide association study identifies 112 new loci for body mass index in the Japanese population. Nat Genet. 2017;49(10):1458-67; doi:10.1038/ng.3951.
30. Kanai M, Akiyama M, Takahashi A, Matoba N, Momozawa Y, Ikeda M, et al. Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases. Nat Genet. 2018;50(3):390-400; doi:10.1038/s41588-018-0047-6.
31. Matoba N, Akiyama M, Ishigaki K, Kanai M, Takahashi A, Momozawa Y, et al. GWAS of smoking behaviour in 165,436 Japanese people reveals seven new loci and shared genetic architecture. Nat Hum Behav. 2019;3(5):471-7; doi:10.1038/s41562-019-0557-y.
32. Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature. 2014;506(7488):376-81; doi:10.1038/nature12873.
33. Lu XF, Wang LY, Chen SF, He L, Yang XL, Shi YY, et al. Genome-wide association study in Han Chinese identifies four new susceptibility loci for coronary artery disease. Nature Genetics. 2012;44(8):890-+; doi:10.1038/ng.2337.
34. Marzec J, Mao X, Li M, Wang M, Feng N, Gou X, et al. A genetic study and meta-analysis of the genetic predisposition of prostate cancer in a Chinese population. Oncotarget. 2016;7(16):21393-403; doi:10.18632/oncotarget.7250.
35. Okada Y, Sim X, Go MJ, Wu JY, Gu D, Takeuchi F, et al. Meta-analysis identifies multiple loci associated with kidney function-related traits in east Asian populations. Nat Genet. 2012;44(8):904-9; doi:10.1038/ng.2352.
36. Zeng C, Matsuda K, Jia WH, Chang J, Kweon SS, Xiang YB, et al. Identification of Susceptibility Loci and Genes for Colorectal Cancer Risk. Gastroenterology. 2016;150(7):1633-45; doi:10.1053/j.gastro.2016.02.076.
37. Zhou X, Chen Y, Mok KY, Zhao Q, Chen K, Chen Y, et al. Identification of genetic risk factors in the Chinese population implicates a role of immune system in Alzheimer's disease pathogenesis. Proceedings of the National Academy of Sciences. 2018;115(8):1697; doi:10.1073/pnas.1715554115.
38. Gan W, Walters RG, Holmes MV, Bragg F, Millwood IY, Banasik K, Chen Y, Du H, Iona A, Mahajan A, et al: Evaluation of type 2 diabetes genetic risk variants in Chinese adults: findings from 93,000 individuals from the China Kadoorie Biobank. Diabetologia. 2016;59(7):1446-1457. doi:10.1007/s00125-016-3920-9
39. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology. 2013;31:814;

doi:10.1038/nbt.2676.
40. Canga Y, Emre A, Yuksel GA, Karatas MB, Yelgec NS, Gurkan U, et al. Assessment of Atrial Conduction Times in Patients with Newly Diagnosed Parkinson's Disease. Parkinsons Dis. 2018;2018:2916905; doi:10.1155/2018/2916905.
41. Ihara M, Washida K. Linking Atrial Fibrillation with Alzheimer's Disease: Epidemiological, Pathological, and Mechanistic Evidence. J Alzheimers Dis. 2018;62(1):61-72; doi:10.3233/JAD-170970.
42. Conen D, Wong JA, Sandhu RK, Cook NR, Lee I-M, Buring JE, et al. Risk of Malignant Cancer Among Women With New-Onset Atrial Fibrillation. JAMA Cardiology. 2016;1(4):389-96; doi:10.1001/jamacardio.2016.0280.
43. Rousseeuw PJ. Silhouettes: A graphical aid to the interpretation and validation of cluster analysis. Journal of Computational and Applied Mathematics. 1987;20:53-65; doi:https://doi.org/10.1016/0377-0427(87)90125-7.
44. Blekhman R, Goodrich JK, Huang K, Sun Q, Bukowski R, Bell JT, et al. Host genetic variation impacts microbiome composition across human body sites. Genome Biol. 2015;16:191; doi:10.1186/s13059-015-0759-1.
45. Goodrich JK, Davenport ER, Clark AG, Ley RE. The Relationship Between the Human Genome and Microbiome Comes into View. Annu Rev Genet. 2017;51:413-33; doi:10.1146/annurev-genet-110711-155532.
46. Kuehbacher T, Rehman A, Lepage P, Hellmig S, Fölsch UR, Schreiber S, et al. Intestinal TM7 bacterial phylogenies in active inflammatory bowel disease. Journal of Medical Microbiology. 2008;57(12):1569-76; doi:https://doi.org/10.1099/jmm.0.47719-0.
47. He X, McLean JS, Edlund A, Yooseph S, Hall AP, Liu S-Y, et al. Cultivation of a human-associated TM7 phylotype reveals a reduced genome and epibiotic parasitic lifestyle. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(1):244-9; doi:10.1073/pnas.1419038112.
48. Bor B, Bedree JK, Shi W, McLean JS, He X. Saccharibacteria (TM7) in the Human Oral Microbiome. Journal of Dental Research. 2019;98(5):500-9; doi:10.1177/0022034519831671.
49. Cirulli ET, Goldstein DB. Uncovering the roles of rare variants in common disease through whole-genome sequencing. Nat Rev Genet. 2010;11(6):415-25; doi:10.1038/nrg2779.
50. Vinter N, Christesen Amanda MS, Fenger-Grøn M, Tjønneland A, Frost L. Atrial Fibrillation and Risk of Cancer: A Danish Population-Based Cohort Study. Journal of the American Heart Association. 2018; doi:10.1161/JAHA.118.009543.
51. Zoja C, Corna D, Rottoli D, Zanchi C, Abbate M, Remuzzi G. Imatinib ameliorates renal disease and survival in murine lupus autoimmune disease. Kidney International. 2006;70(1):97-103; doi:https://doi.org/10.1038/sj.ki.5001528.
52. Boursi B, Mamtani R, Haynes K, Yang Y-X. Parkinson's disease and colorectal cancer risk-A nested case control study. Cancer Epidemiol. 2016;43:9-14;

doi:10.1016/j.canep.2016.05.007.
53. Xie X, Luo X, Xie M. Association between Parkinson's disease and risk of colorectal cancer. Parkinsonism & Related Disorders. 2017;35:42-7; doi:10.1016/j.parkreldis.2016.11.011.
54. van Rheenen W, Shatunov A, Dekker AM, McLaughlin RL, Diekstra FP, Pulit SL, et al. Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis. Nat Genet. 2016;48(9):1043-8; doi:10.1038/ng.3622.
55. Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet. 2013;45(12):1452-8; doi:10.1038/ng.2802.
56. Pankratz N, Beecham GW, DeStefano AL, Dawson TM, Doheny KF, Factor SA, et al. Meta-analysis of Parkinson's disease: identification of a novel locus, RIT2. Ann Neurol. 2012;71(3):370-84; doi:10.1002/ana.22687.
57. Zhao L, Wang G, Siegel P, He C, Wang H, Zhao W, et al. Quantitative genetic background of the host influences gut microbiomes in chickens. Sci Rep. 2013;3:1163-; doi:10.1038/srep01163.
58. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. American journal of human genetics. 2007;81(3):559-75; doi:10.1086/519795.
59. Anderson CA, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT. Data quality control in genetic case-control association studies. Nat Protoc. 2010;5(9):1564-73; doi:10.1038/nprot.2010.116.
60. Delaneau O, Marchini J, Zagury J-F. A linear complexity phasing method for thousands of genomes. Nature Methods. 2011;9:179; doi:10.1038/nmeth.1785.
61. Delaneau O, Marchini J, The Genomes Project C, McVean GA, Donnelly P, Lunter G, et al. Integrating sequence and array data to create an improved 1000 Genomes Project haplotype reference panel. Nature Communications. 2014;5:3934; doi:10.1038/ncomms4934.
62. Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. Nature Genetics. 2016;48:1284; doi:10.1038/ng.3656.
63. Clarke L, Fairley S, Zheng-Bradley X, Streeter I, Perry E, Lowy E, et al. The international Genome sample resource (IGSR): A worldwide collection of genome variation incorporating the 1000 Genomes Project data. Nucleic Acids Research. 2016; doi:10.1093/nar/gkw829.
64. Okada Y, Kim K, Han B, Pillai NE, Ong RT, Saw WY, et al. Risk for ACPA-positive rheumatoid arthritis is driven by shared HLA amino acid polymorphisms in Asian and European populations. Hum Mol Genet. 2014;23(25):6916-26; doi:10.1093/hmg/ddu387.
65. Pillai NE, Okada Y, Saw WY, Ong RT, Wang X, Tantoso E, et al. Predicting HLA alleles from high-resolution SNP data in three Southeast Asian populations. Hum Mol Genet. 2014;23(16):4443-51;

doi:10.1093/hmg/ddu149.
66. Jia X, Han B, Onengut-Gumuscu S, Chen W-M, Concannon PJ, Rich SS, et al. Imputing Amino Acid Polymorphisms in Human Leukocyte Antigens. PLOS ONE. 2013;8(6):e64683; doi:10.1371/journal.pone.0064683.
67. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26(19):2460-1; doi:10.1093/bioinformatics/btq461.
68. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013;10(10):996-8; doi:10.1038/nmeth.2604.
69. Second Genome, Inc: the Greengenes Databases.http://greengenes.secondgenome.com/.Accessed 12 Mar 2019.
70. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335-6; doi:10.1038/nmeth.f.303.
71. Lee SH, Wray NR, Goddard ME, Visscher PM. Estimating missing heritability for disease from genome-wide association studies. Am J Hum Genet. 2011;88(3):294-305; doi:10.1016/j.ajhg.2011.02.002.
72. Visscher PM, Hemani G, Vinkhuyzen AAE, Chen G-B, Lee SH, Wray NR, et al. Statistical Power to Detect Genetic (Co)Variance of Complex Traits Using SNP Data in Unrelated Samples. PLOS Genetics. 2014;10(4):e1004269; doi:10.1371/journal.pgen.1004269.
73. Caliński T, Harabasz J. A dendrite method for cluster analysis. Communications in Statistics. 1974;3(1):1-27; doi:10.1080/03610927408827101.
74. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. Am J Hum Genet. 2011;88(1):76-82; doi:10.1016/j.ajhg.2010.11.011.
75. Yang J, Zaitlen NA, Goddard ME, Visscher PM, Price AL. Advantages and pitfalls in the application of mixed-model association methods. Nat Genet. 2014;46(2):100-6; doi:10.1038/ng.2876.
76. Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, et al. Common SNPs explain a large proportion of the heritability for human height. Nature Genetics. 2010;42:565; doi:10.1038/ng.608.
77. Bulik-Sullivan BK, Loh P-R, Finucane HK, Ripke S, Yang J, Patterson N, et al. LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. Nature Genetics. 2015;47(3):291-5; doi:10.1038/ng.3211.
78. Lee SH, Yang J, Goddard ME, Visscher PM, Wray NR. Estimation of pleiotropy between complex diseases using single-nucleotide polymorphism-derived genomic relationships and restricted maximum likelihood. Bioinformatics. 2012;28(19):2540-2; doi:10.1093/bioinformatics/bts474.
79. Wang H, Zhang F, Zeng J, Wu Y, Kemper KE, Xue A, et al. Genotype-by-environment interactions inferred from genetic effects on phenotypic variability in the UK Biobank. Science Advances.

2019;5(8):eaaw3538; doi:10.1126/sciadv.aaw3538.
80. Bretherton CS, Widmann M, Dymnikov VP, Wallace JM, Bladé I. The Effective Number of Spatial Degrees of Freedom of a Time-Varying Field. Journal of Climate. 1999;12(7):1990-2009; doi:10.1175/1520-0442(1999)012<1990:Tenosd>2.0.Co;2.
81. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. Genet Epidemiol. 2016;40(4):304-14; doi:10.1002/gepi.21965.
82. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. Eur J Epidemiol. 2017;32(5):377-89; doi:10.1007/s10654-017-0255-x.
83. Verbanck M, Chen C-Y, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nature Genetics. 2018;50(5):693-8; doi:10.1038/s41588-018-0099-7.
84. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28(1):27-30; doi:10.1093/nar/28.1.27.
85. Kanehisa M, Sato Y, Furumichi M, Morishima K, Tanabe M. New approach for understanding genome variations in KEGG. Nucleic Acids Res. 2019; doi:10.1093/nar/gky962.
86. Kanehisa M. Toward understanding the origin and evolution of cellular organisms. Protein Sci. 2019; doi:10.1002/pro.3715.
87. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-504; doi:10.1101/gr.1239303.
88. Kurtz ZD, Müller CL, Miraldi ER, Littman DR, Blaser MJ, Bonneau RA. Sparse and Compositionally Robust Inference of Microbial Ecological Networks. PLOS Computational Biology. 2015; doi:10.1371/journal.pcbi.1004226.
89. Reynolds AP, Richards G, de la Iglesia B, Rayward-Smith VJ. Clustering Rules: A Comparison of Partitioning and Hierarchical Clustering Algorithms. Journal of Mathematical Modelling and Algorithms. 2006;5(4):475-504; doi:10.1007/s10852-005-9022-1.
90. Kaufman L, Rousseeuw P. Partitioning Around Medoids (Program PAM). John Wiley & Sons, Inc; 1990. p. 68-125.

Figure legends

Figure 1 Study overview. The figure shows the highlights of our study. First, we performed a microbiome genome-wide association study in a Chinese population (Step A). We validated significant genetic variants reported in the previous studies and replicated our results in an independent cohort. Second, we investigated the causal relationship between gut microbiome and human complex diseases, using host genetics as instrumental variables for the bi-directional Mendelian randomization (MR) analysis (Step B). For the analysis of gut microbiome on complex traits, we used public available GWAS summary statistics of complex traits (n=58) and diseases (type 2 diabetes mellitus (T2DM), atrial fibrillation (AF), colorectal cancer (CRC) and rheumatoid arthritis) reported by BioBank Japan [27-32]. For the reserve MR analyses, the diseases of interests included T2DM (cases: 7,109; non-cases: 86,022), AF (cases: 8,180; non-cases: 28,612), coronary artery disease (cases: 1,515; non-cases: 5,019), chronic kidney disease (n=71,149), Alzheimer's disease (cases: 477; non-cases: 442), CRC (cases: 8,027; non-cases: 22,577) and prostatic cancer (cases: 495; non-cases: 640) reported in the previous large-scale GWAS in East Asians [27, 33-38]. Finally, we identified common and distinct gut microbiome features across different diseases (Step C).

Figure 2 The SNP-based heritability of gut microbiome. The plot shows the taxa with nominally significant heritability estimates ($p < 0.05$). * $p < 0.05/n$, n is the effective number of independent taxa on each taxonomy level.

Figure 3 Effect of host genetically predicted higher atrial fibrillation risk on gut microbiome. (A). Causal association of atrial fibrillation with abundance of *Burkholderiales*, *Alcaligenaceae*, *Lachnobacterium* and *Bacteroides coprophilus*. The effect sizes of atrial fibrillation on taxa are changes in abundance of bacteria (1-SD of log-transformed) per genetically determined higher log odds of atrial fibrillation. **(B)**. Causal association of atrial fibrillation with presence of *Barnesiellaceae*, *Veillonellaceae_undefined* and *Mitsuokella*. The effect size of atrial fibrillation on taxa are present as odds ratio increase in log odds of atrial fibrillation.

Figure 4 Association and cluster of diseases predicted by the gut microbiome. (A). Plot of clusters in Guangzhou Nutrition and Health Study (GNHS) cohort (n=1919). **(B)**. Plot of cluster results in the replication cohort (n=217). **(C)**. Plot of 5 clusters in antibiotic-taking participants (n=18). The optimal cluster is 5 in GNHS cohort and 6 in the replication. The clusters share consistent components between two studies. In contrast, components are different between antibiotic-taking participants and control groups. Dimension1 (Dim1) and dimension2 (Dim2) can explain 40.1% and 13.1% variance, respectively in GNHS cohort. The annotation for variables is as following. AT: African trypanosomiasis, AD: Alzheimer's disease, V1: Amoebiasis, ALS: Amyotrophic lateral sclerosis, BC: Bladder cancer, CD: Chagas disease, CML: Chronic myeloid leukemia, CRC: Colorectal cancer, V2: Hepatitis C, HD:

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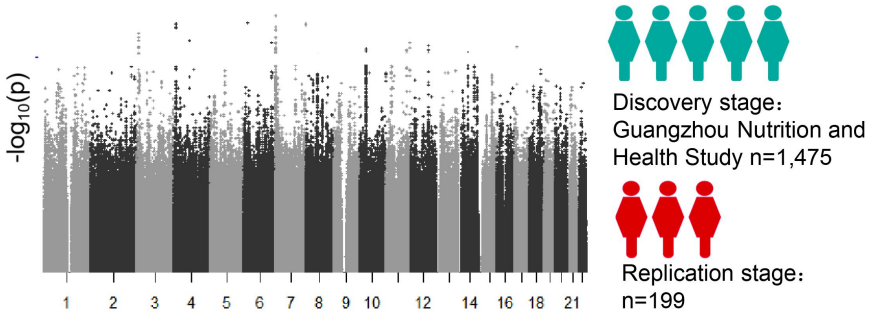
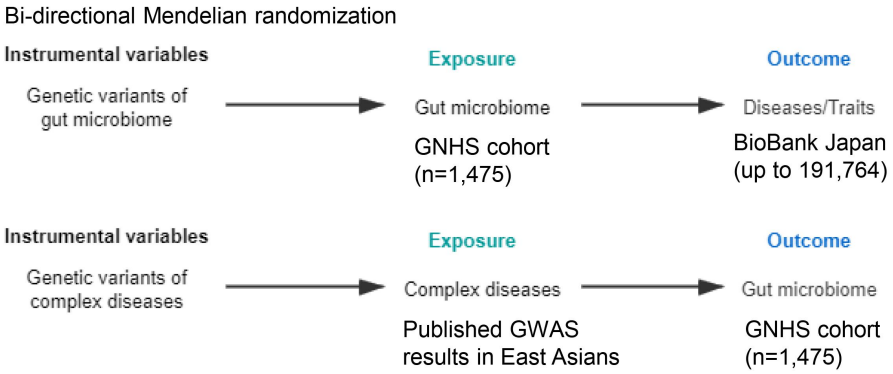
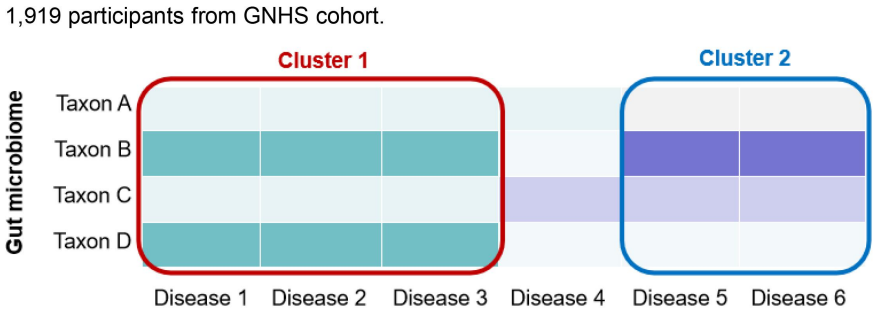
A. Association of host genetics with gut microbiome in a Chinese population.



B. The causal relationships between gut microbiome and human complex diseases.



C. The shared and distinct microbiome features among human complex diseases.



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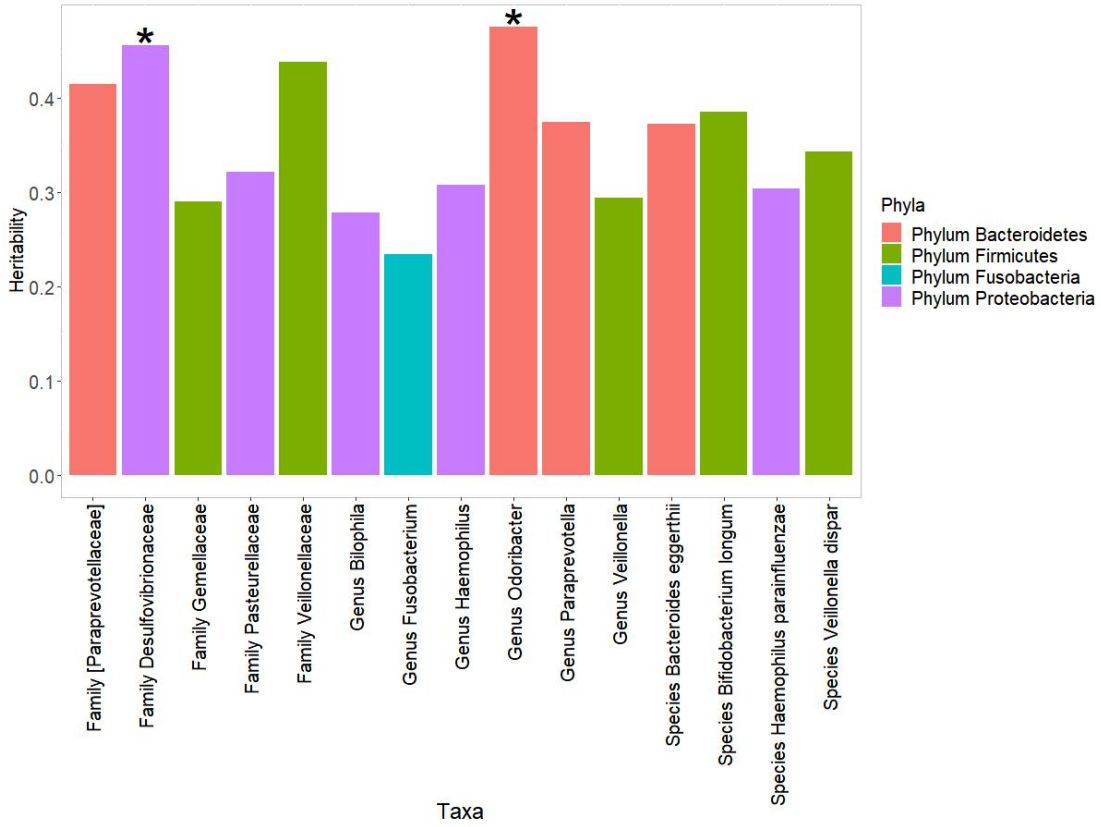

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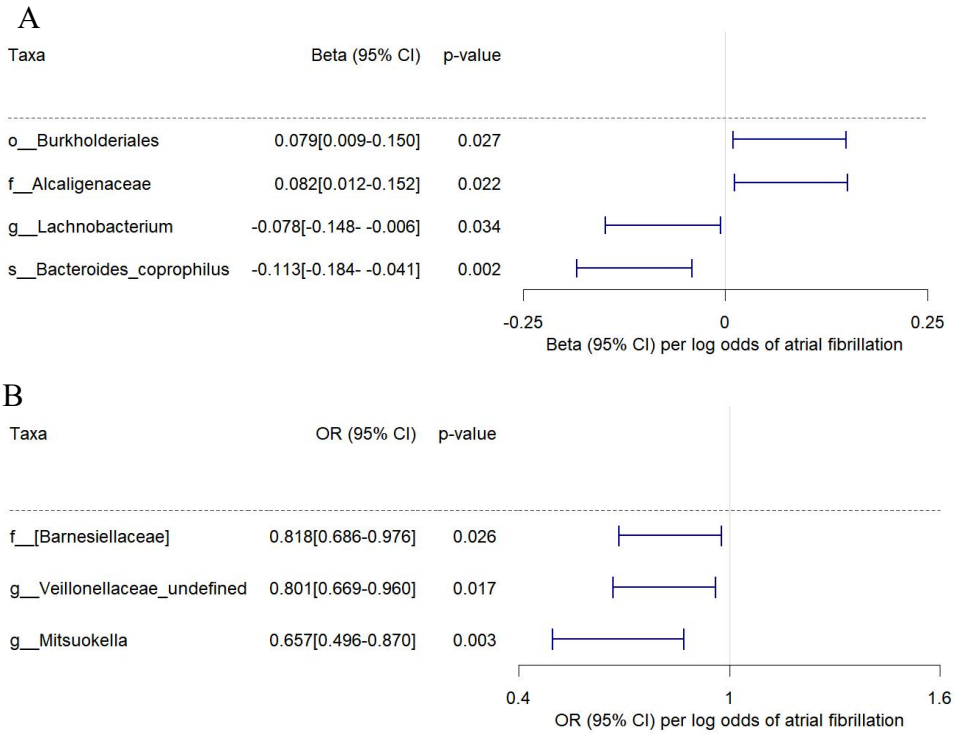

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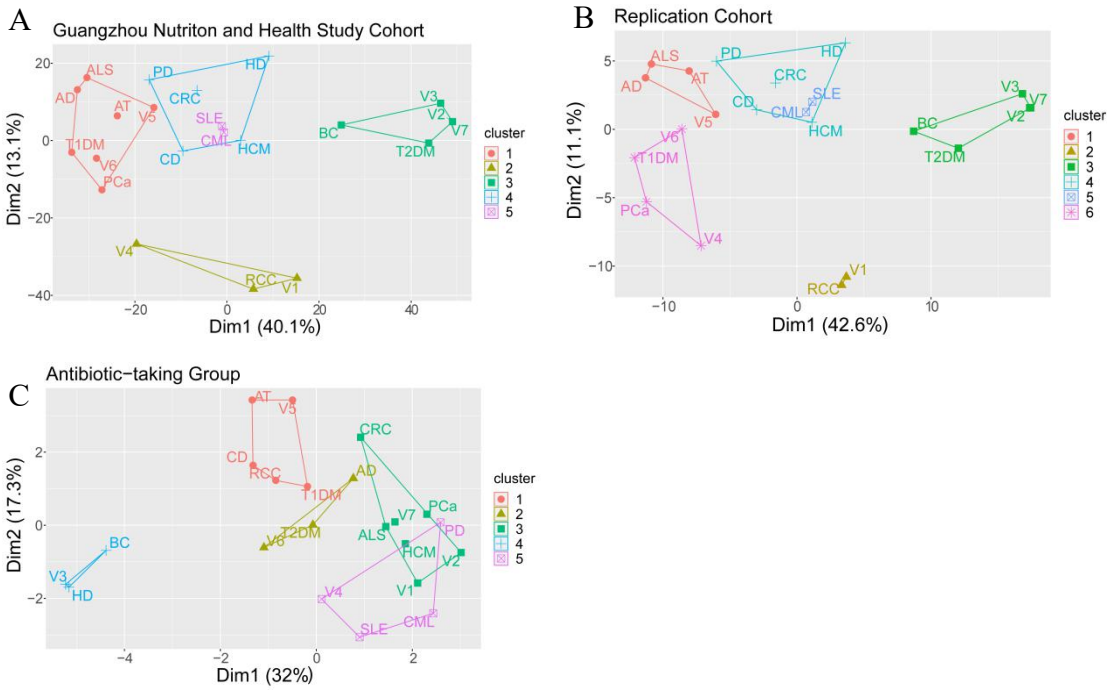

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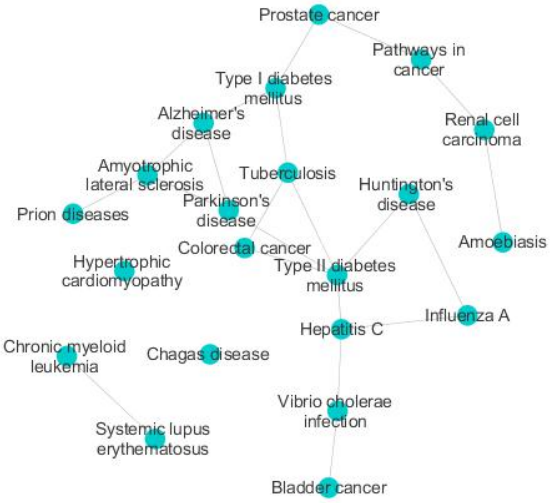
Prostate cancer
Pathways in cancer
Type I diabetes mellitus
Alzheimer's disease
Renal cell carcinoma
Amyotrophic lateral sclerosis
Tuberculosis
Huntington's disease
Prion diseases
Parkinson's disease
Amoebiasis
Colorectal cancer
Hypertrophic cardiomyopathy
Type II diabetes mellitus
Influenza A
Hepatitis C
Chronic myeloid leukemia
Chagas disease
Vibrio cholerae infection
Systemic lupus erythematosus
Bladder cancer

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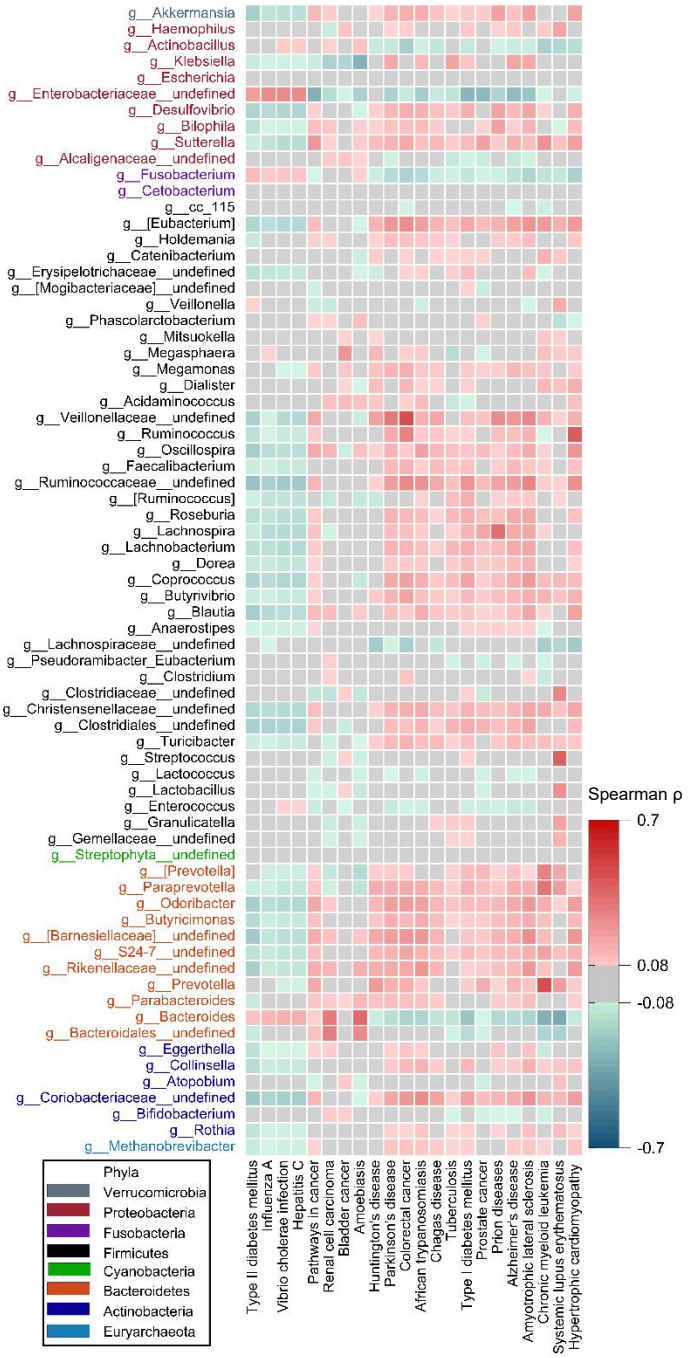

Supplementary Tables *[Supplementary Tables.xls]*

Supplementary Table S1 Transformation of traits in BioBank Japan and taxa in GNHS
Supplementary Table S2 Required effect size (beta) to reach 80% of power in GNHS cohort
Supplementary Table S3 Heritability of taxa, enterotype and alpha diversity
Supplementary Table S4 Significant genetic correlations of gut microbiome and metabolic traits
Supplementary Table S5 Significant associations of all taxa with SNPs identified in the discovery stage before adjustment(p<5e-8)
Supplementary Table S6 Replication of genetic variants associated with taxa
Supplementary Table S7 Replication of genetic variants associated with beta diversity
Supplementary Table S8 Replication of genetic variants associated with alpha diversity
Supplementary Table S9 Lead SNPs used to construct polygenic scores
Supplementary Table S10 MR analysis of gut microbiota on traits and diseases
Supplementary Table S11 MR analysis of diseases on gut microbiota features
Supplementary Table S12 Spearman's correlation of certain taxa and complex diseases
Supplementary Table S13 Spearman ' s correlation of gut microbiota on genus level and characteristics

Supplementary Figures *[Supplementary Figures.pdf]*

Supplementary Figure 1 Genome-wide analysis results of taxa.
Supplementary Figure 2 Spearman's correlation of the relative abundance of AF-associated taxa with the relative level of diseases predicted by PICRUSt.