Mapping the gut microecological multi-omics signatures to serum metabolome and their impact on cardiometabolic health in elderly adults

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Summary

Background Mapping gut microecological features to serum metabolites (SMs) will help identify functional links between gut microbiome and cardiometabolic health.

Methods This study encompassed 836-1021 adults over 9.7 year in a cohort, assessing metabolic syndrome (MS), carotid atherosclerotic plaque (CAP), and other metadata triennially. We analyzed mid-term microbial metagenomics, targeted fecal and serum metabolomics, host genetics, and serum proteomics.

Findings Gut microbiota and metabolites (GMM) accounted for 15.1% overall variance in 168 SMs, with individual GMM factors explaining 5.65%–10.1%, host genetics 3.23%, and sociodemographic factors 5.95%. Specifically, GMM elucidated 5.5%–49.6% variance in the top 32 GMM-explained SMs. Each 20% increase in the 32 metabolite score (derived from the 32 SMs) correlated with 73% (95% confidence interval [CI]: 53%–95%) and 19% (95% CI: 11%–27%) increases in MS and CAP incidences, respectively. Among the 32 GMM-explained SMs, sebacic acid, indoleacetic acid, and eicosapentaenoic acid were linked to MS or CAP incidence. Serum proteomics revealed certain proteins, particularly the apolipoprotein family, mediated the relationship between GMM-SMs and cardiometabolic risks.

Interpretation This study reveals the significant influence of GMM on SM profiles and illustrates the intricate connections between GMM-explained SMs, serum proteins, and the incidence of MS and CAP, providing insights into the roles of gut dysbiosis in cardiometabolic health via regulating blood metabolites.

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Keywords: Gut microbiota; Metabolomics; Proteomics; Multi-omics; Cardiometabolic health

Introduction

Cardiovascular diseases (CVDs), the primary cause of death worldwide, result in approximately 17.9 million fatalities annually.[1] Modifiable risk factors like atherosclerosis, metabolic syndrome (MS), along with related intestinal microecology and host metabolic traits, play a

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Research in context

Evidence before this study

Recent studies increasingly recognize the gut microbiome's crucial role in cardiometabolic health, primarily through its influence on blood metabolites. However, human research on how microbial features contribute to the blood metabolome has been limited and yielded mixed results. The link between gut microbiome-associated serum metabolites (SMs), cardiovascular diseases, and intermediary blood proteins remains unclear.

Added value of this study

This extensive longitudinal study demonstrates that gut microbiota and metabolites (GMM) — encompassing species, MetaCyc pathways, and fecal metabolome — accounts for significant variance in total and 32 individual SM profiles. Several SMs (like oxoglutaric acid, citramalic acid, eicosapentaenoic acid, indoleacetic acid, suberic acid, chenodeoxycholic acid, glycoursodeoxycholic acid, and sebacic acid, and the combined 32-SM score) have been identified as crucial SMs linking GMM and the incidence of metabolic syndrome and carotid atherosclerotic plaque. Serum proteomics revealed that certain proteins, particularly the apolipoprotein family, mediated the relationship between GMM-SMs and cardiometabolic risks.

Implications of all the available evidence

Our findings emphasize GMM's influence on SMs in the elderly and its potential in modulating blood metabolites and proteins for cardiovascular disease prevention. The identification of specific SMs that connect gut microecology and serum proteins with cardiometabolic health provides valuable evidence for future intervention strategies and elucidating the microbiota's role in cardiometabolic health.

crucial role in the risk of CVDs.[2] A deeper understanding of the basis of these cardiometabolic traits can shed light on the prevention, diagnosis, and treatment of CVD.

Recent research increasingly underscores the pivotal role of gut microbiome and related microecology in human cardiometabolic health.[3] This influence largely hinges on a diverse array of metabolites produced by gut microbiota.[4] Many individual serum metabolites (SMs) originating from gut microbiota, such as short-chain fatty acids (SCFAs), trimethylamine oxide, uremic toxins, bile acids, anthocyanins, and lipopolysaccharides, have been identified as potential influencers of CVD risk.[5] These findings highlight the gut microbiota's capacity to impact cardiometabolic health via various blood metabolites.

To date, human studies investigating the contribution of microbial features to the blood metabolome have been limited and yielded inconsistent results.[6,7] For example, in an Israeli cohort of 491 individuals, microbial taxa were found to explain over 40% of the variance in 10 blood metabolites, including quinate.[6] A Dutch study involving 1368 participants revealed that the top five plasma metabolites driven by the gut microbiome (species and pathways) were p-cresol, sulfurous acid, p-cresol sulfate, acetyl-N-formyl-5-methoxy kynurenamine, and 2,3-diketogulonate.[7] A recent study suggests a significant correlation between the gut fecal metabolites and microbial composition, accounting for 67.7% of the variance.[8] However, differences in associations between microbial taxonomic composition and their pathways with metabolites, depending on whether fecal or blood metabolites are used,[9] indicate a need for more detailed investigation into their specific roles in the relationships between gut microbiota and cardiometabolic diseases. To our knowledge, however, no research has yet examined the contribution of fecal metabolites to variance in SM profile. Given the scarce evidence available, there is also a lack of robust evidence elucidating the extent to which the gut microbiota and metabolites (GMM) explain SMs, especially the fecal metabolites, which is a functioning readout of gut microbiota and can be employed as a middle phenotype moderating host–microbiome interactions.[8,10] Moreover, evidence for a robust relationship between fecal microbiota-associated SMs and CVD and possible intermediate blood proteins has not been identified.

This study elucidated the extent to which overall SM and individual SMs could be explained by GMM, including species, pathways, and fecal metabolites. We focused on identifying the critical contributors within GMM-explained SMs (GMM-SMs) and determined the associations between GMM-SMs and the risks of developing MS and carotid atherosclerotic plaque (CAP). Through untargeted serum proteomics analysis, we also sought to uncover potential serum proteins that may link GMM-SMs and MS/CAP risk in a 9.7-year Chinese cohort population.

Methods

Ethics

The study protocol (No. 2018048) has received approval from the Ethics Committee of the School of Public Health at Sun Yat-sen University. Every participant gave their written informed consent, abiding by the guidelines set forth by the institution.

Study participants and study design

Fig. 1 shows the study design with more details in Supplementary Methods. The research utilized data from the Guangzhou Nutrition and Health Study

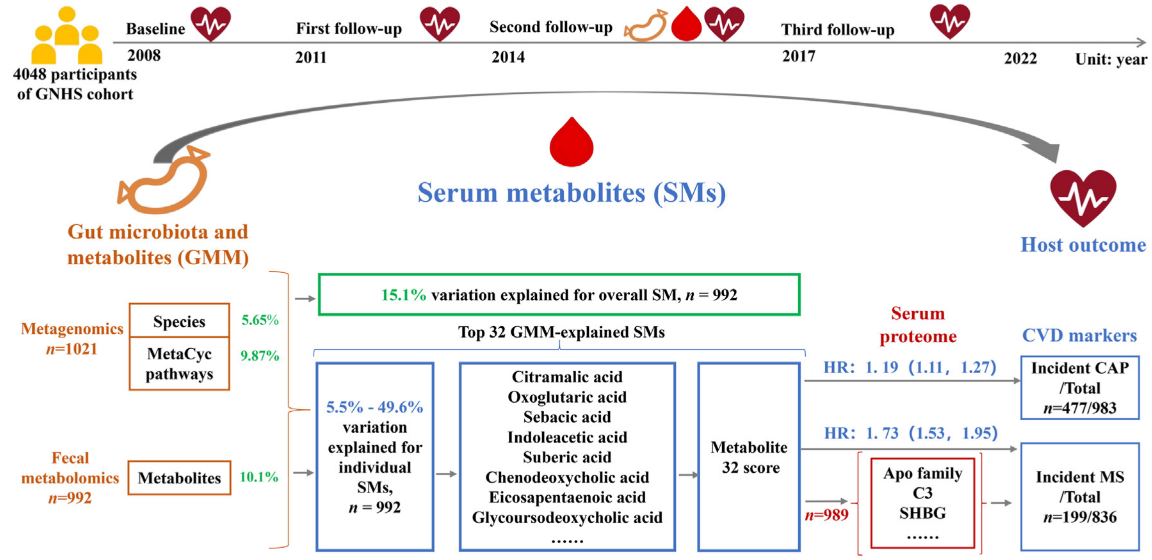

Fig. 1: **Study design and main findings**. Abbreviations: CAP: carotid atherosclerotic plaque; CVD: cardiovascular diseases; GMM: gut microbiota and metabolites; GNHS: Guangzhou Nutrition and Health Study; SMs: serum metabolites; MS: metabolic syndrome; SHBG: sex hormone binding globulin.

(GNHS). This is a population-based prospective study, with the purpose of exploring the influence of nutritional factors on cardiometabolic phenotypes, which is registered at www.clinicaltrials.com under the identifier NCT03179657. Participants were sourced from diverse communities across Guangzhou city (South China) primarily through local advertising, health seminars, and referrals. The study enrolled 4048 healthy individuals aged 40 to 75 from 2008 to 2013 in Guangzhou, China. Data collection occurred triennially from 2008 to 2021. At baseline and during subsequent follow-ups, participants underwent triennial visits to the School of Public Health for direct interviews, physical evaluations, and sample collection. The procedures remained consistent across visits. Detailed information was published in the cohort profile.[11] GNHS data have significantly contributed to understanding the impact of nutrition on metabolic diseases through multi-omic approaches. Multi-omics information of participants was determined using the samples collected during 2015–2019. Finally, this study included 836–1021 participants with data of GMM (microbial species and pathways based on metagenome sequencing of fecal microbiome, and metabolome), host serum metabolomics and proteomics and whole genotyping, and 9.7-year incidence of MS and CAP.

Fecal sample collection procedure

The fecal sample collection followed a standardized protocol. Individuals were instructed to collect fecal specimen if they didn't use any antibiotics and have a trip out of the city in the past 14 days. The characteristics of the stool were also recorded. Collection was performed either on-site or at home using sterile containers with ice. Fecal specimen was immediately transported to the laboratory within 1–2 h. All subsequent processing steps were conducted in a fume hood to maintain an aseptic environment after surface sterilization. The fecal specimens were thoroughly homogenized, aliquoted into 2 ml sterile tubes (each >1.0 g), and stored at a temperature of –80 °C until further analysis.

Blood sample collection procedure

Venous blood samples (about 10 ml) were collected using non-anticoagulant vacuum tubes in the following morning after about 10-h fasting and allowed to coagulate by leaving it at room temperature for 1 h. Serum was then separated into five tubes after centrifuged at 3000 rpm and 4 °C for a duration of 10 min. These aliquots of serum were stored at a temperature of –80 °C until further analysis.

Gut microbiome data analysis

Fecal samples underwent metagenomic sequencing form a library, with each sample being multiplexed and analyzed using an Illumina HiSeq machine following a 150-bp paired-end read protocol. Average 5,835,670 reads per sample were obtained. We applied PRINSEQ v0.20.4 for data quality control. Reads aligned to the human genome (*H. sapiens*, UCSC hg19) were excluded using Bowtie2 v2.2.5. For taxonomic analysis, MetaPhlAn2 v2.6.0 was employed, utilizing a library of clade-specific markers to achieve bacterial quantification at the level of species.[12] The abundance of metabolic pathways was annotated using HUMAnN2, which mapped reads to a functionally annotated pan-genome custom database. The reads were further grouped into 160 species (Table S1) and 448 pathways from the MetaCyc Metabolic Pathways Database (Table S2).

Host genotyping data

Genotyping was performed using the Illumina ASA-750K chip. PLINK1.9[13] software was used for quality control and association screening. Individuals exhibiting extreme heterozygosity, defined as values outside the range of the mean ± 3 SDs, were excluded from the analysis.[14]

Profiling the metabolome in serum and stool specimen

The metabolome in serum and feces were measured employing a targeted metabolomics profiling approach by an ACQUITY UPLC-MS/MS Xevo TQ-S (ultra-high-performance liquid chromatography-tandem mass spectrometry) from Waters Corp., (MA, USA). This approach quantified 199 fecal metabolites and 168 SMs, encompassing a diverse array of compounds, including bile acids, amino acids, benzenes, fatty acids, carbohydrates, carnitines, cinnamic acids, organic acids, indoles, nucleosides, phenylpropanoic acids, organic oxygen compounds, pyridines, peptidomimetics, and pyrroles (Tables S3 and S4).

Measurement of serum proteomics

After sample preparation, a liquid chromatography coupled to tandem mass spectrometry system (Eksigent NanoLC 400, Dublin, CA, USA) were analyzed peptide samples. The system was coupled with a Triple TOF 5600 system (SCIEX, CA, USA). Data acquisition was conducted using the Sequential Window Acquisition of All Theoretical Fragment Ions-Mass Spectrum (SWATH-MS) technique.[15] Analysis with OpenSWATH (2.1)[16] against a pan-human spectral library[17] annotated 438 serum proteins.

Diagnostic criteria for incident cardiometabolic diseases

MS was defined according to the Third Edition Asian Chinese criteria from the National Cholesterol Education Program Adult Treatment Panel.[18] CAP was diagnosed by a carotid intima-media thickness (cIMT) equal to or greater than 1.5 mm in accordance with the European Mannheim Consensus.[19] Incident MS or CAP was identified in participants who were free of these conditions at baseline but developed them during the 9.7 follow-up period.

Statistical analysis

Data preparation

Listwise deletion was employed for missing data in the primary variables–GMM, SM metrics, and cardiometabolic incidences. In contrast, median imputation was utilized for serum proteomics data, pertained to secondary research objectives. For covariates with missing values, data from adjacent follow-ups were imputed. Regarding the omics data for GMM, we conducted non-parametric analyses after transforming the raw data to relative abundance or concentration. Both SMs and proteomic data were standardized to Z-scores for linear regression analysis and categorized into gender-specific quintiles for Cox regression analysis.

Mapping explanations of GMM and host factors for overall SM variations

To estimate the contribution of GMM and host factors on overall variance in SM profiles, we employed feature selection through permutation multivariate analysis of variance (PERMANOVA) based on Bray–Curtis distances using R “vegan” package, with 999 permutations.[20] Initially, GMM factors (species, pathways, and fecal metabolites) were analyzed separately to estimate their contribution to overall inter-individual SM variations, retaining only those with a permutational $P < 0.05$. Genetic variants were included based on single nucleotide polymorphisms (SNPs) associated with SMs. A combined PERMANOVA model then estimated the total contribution of all features.

Mapping explanations of GMM for individual SM variations

To elucidate the collective influence of GMM on individual SMs and identify key GMM contributing factors, we used random forest algorithm, utilizing the ‘randomForest’ R package (ntree = 1000, mtry = the number of predictor features/3). This approach regressed the species, pathways, and fecal metabolites against SM quintiles. Over 100 iterations, we ranked variables based on feature importance, which was determined by the difference in Mean Squared Error (MSE, post-shuffle MSE minus original MSE). The most critical GMM features were those causing the highest increase in MSE.

Associations of GMM-related SMs with incident CAP and MS risks

Cox regression analysis was employed to determine the association between the top 32 GMM-explained SMs and the risks of incident MS and CAP, adjusted for sex, education, age, smokers, household income, marital status, tea drinkers, alcohol drinkers, physical activity, multivitamin use, daily energy intake and other significant SMs with step-wise variable selection (*SPSS* 12, *SPSS* Inc.). This analysis led to the creation of the 32 metabolite score (32-SMS). This score, calculated for each individual, aggregates the quintile values of serum metabolites, adjusted in accordance with the β coefficient of their association with incident MS. The associations of 32-SMS with the risk of MS and CAP were also calculated after further adjusting for BMI for sensitivity analyses.

Restricted cubic spline (RCS) analyses (R package 'rms', knots = 4) were utilized to flexibly model the associations of the 32-SMS and crucial individual metabolites (identified by a P-value <0.01 in the above Cox regression) with incident MS or CAP risks.

Association of SMs, serum proteins and incident cardiometabolic diseases

Linear regression model was employed to detect the association of SMs with serum proteins, verifying model assumptions (linearity, independence, normality, and homoscedasticity) through residual and predicted value plots. Cox regression, utilizing R packages 'survival' and 'MASS', was utilized to assess the link between serum proteins and incident cardiometabolic diseases, with scaled Schoenfeld residuals employed to confirm the proportional hazards assumption.

Visualizing the relationship of GMM, SMs and metabolic traits with a network

In order to visualize GMM-SM-cardiometabolic trait relationships, we plotted the network, which was constructed according to the Spearman coefficients higher than 0.12 (all $P < 0.00015$). The network was visualized using the program Cytoscape.[21]

Role of funders

Funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish results.

Results

Participants' characteristics

Numbers of individuals at each part of the study were reported in Fig. 1. This study encompassed 836 to 1021 participants with data related to GMM, host serum metabolomics and proteomics, along with whole genotyping. Additionally, we recorded a 9.7-year incidence of MS (199 diagnosed) and CAP (477 diagnosed). Of the 992 participants with serum metabolome included in the study, 311 were male and 681 were female. Those participants with higher 32-SMS tended to be older and exhibited higher BMI (Table S5).

The variances of overall serum metabolome explained by gut microbiome and metabolome

In assessing the relative contributions of host characteristics, genetics, and GMM to the serum metabolome, we quantified the variance attributable to these factors in the overall SM profile. Through pairwise association analysis between each SMs and genetic variants, thirteen unique genetic variants were identified (Table S6). Separate PERMANOVA models were then used to evaluate the contribution of each microecological feature type to the overall SM, including only features with $P < 0.05$ in the feature selection phage. These analyses identified 12 species (Table S7), 26 pathways (Table S8), and 45 fecal metabolites (Table S9).

The variance (R^2) in SM profiles was explained by species (5.65%), pathways (9.87%), fecal metabolites (10.1%), and combined GMM factors (15.1%), as shown in Table S10. These percentages surpassed those explained by host demographic and socioeconomic factors (5.95%) and genetics (3.23%). Collectively, these factors accounted for 25.3% of the total variance in the whole serum metabolome (Fig. 2A, Table S11). The top five contributors of each category including the following species (*Bifidobacterium longum, Clostridium nexile, Megamonas hypermegale, Faecalibacterium prausnitzii,* and *Klebsiella unclassified*), pathways (benzoyl-CoA degradation I [PWY1361], 4-deoxy-L-threo-hex-4-enopyranuronate degradation [PWY6507], l-arginine biosynthesis II [ARGSYNBSUBPWY], and superpathways of heme biosynthesis from glutamate [PWY5918] and glycine [PWY5920]), and fecal metabolites (2-phenylpropionate, rhamnose, L-histidine, L-leucine, and L-glutamic acid), as detailed in (Fig. 2B–D).

Variances in individual serum metabolites explained by gut microbiota and metabolites

Analysis of 168 individual SMs revealed that GMM accounted for 5.5%–49.6% variance in 32 key metabolites (Fig. 2E). The top five among these SMs were glycolithocholic acid 3-sulfate (3S-GLCA), 2-phenylpropionate, hydrocinnamic acid, glycodeoxycholic acid, and deoxycholic acid (Fig. S1A and Table S12). Among the three GMM categories (species, pathways, and fecal metabolites), random forest models revealed that fecal metabolites were the primary influencers for the majority of these SMs, particularly fecal 2-phenylpropionate, 7-ketodeoxycholic acid (7-DHCA), hydrocinnamic acid, lithocholic acid, and phenylacetic acid (Fig. S1A). Notably, *Eubacterium hallii* stood out as a critical determinant for eight specific SMs (e.g., glyceric acid, suberic acid, and adipic acid) (Fig. S1A and Table S12). The most five influential factors in the three GMM categories for these 32 SMs were further delineated (Fig. S1B–F and Table S12).

Serum metabolites, CVD-related proteins, and cardiovascular diseases or risk factors

Heatmap and forest charts showed associations of the top 32 GMM-SMs with CVD-related proteins, CVD risk factors, and incident cardiometabolic diseases (Fig. 3A). The 32-SMS, derived from these GMM-SMs, exhibited an inverse association with HDL cholesterol, whereas tended to be positively correlate with other cardiometabolic traits. Cox regression and restricted cubic spline analyses indicated significant linear or nonlinear associations between SM concentrations and the risk of cardiometabolic diseases (MS and CAP). Notably, 32-SMS (unadjusted HR 1.77 and 1.20, respectively, and

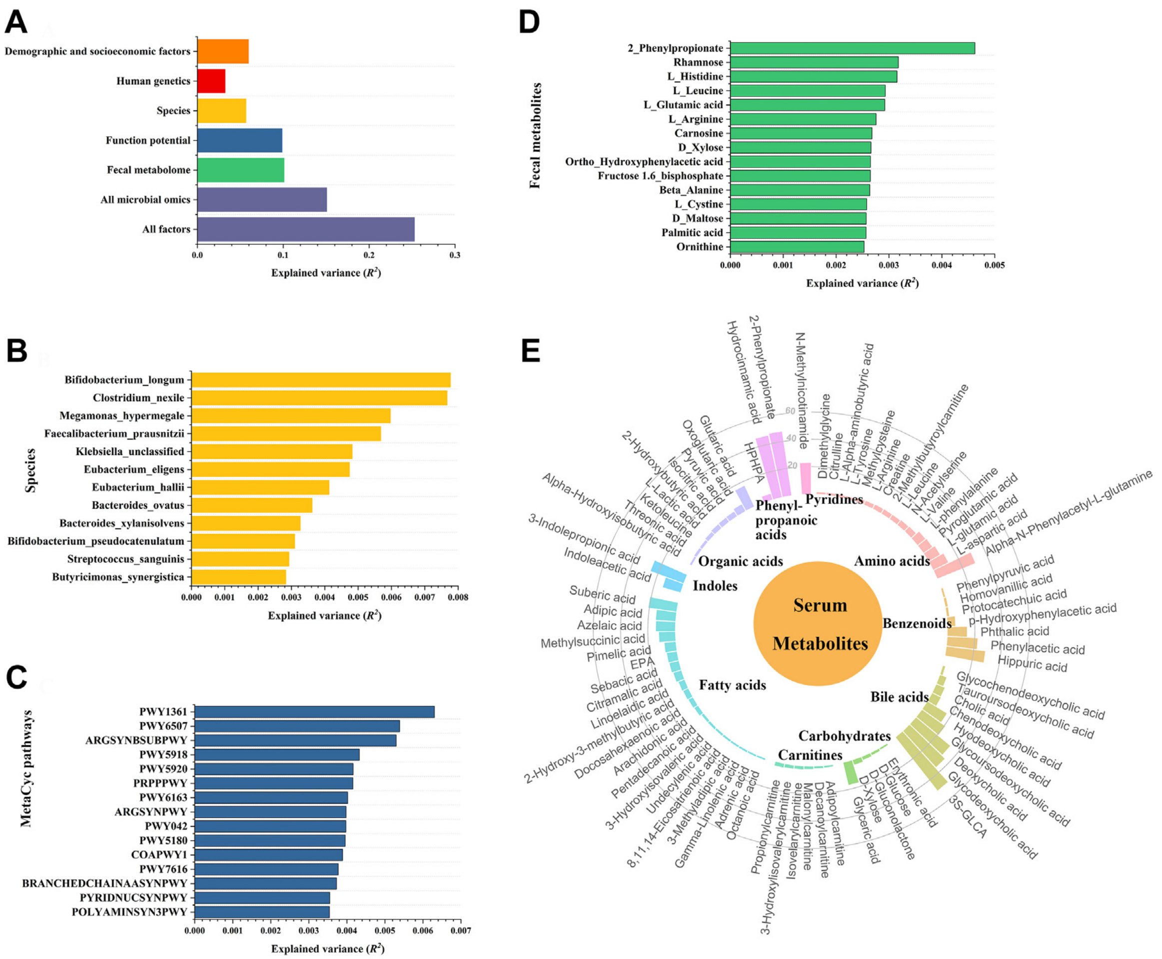

***Fig. 2:* Contribution of host and gut microbial factors to the variances of serum metabolome**. (A) The contribution of host and gut microecological features on serum metabolite profile. This result came from permutational multivariate analysis of variance (PERMANOVA) of overall serum metabolite levels based on separate models for each feature group and combined models. The bars for each group represent the cumulative explained variance of each group of features. (B–D) The variance in serum metabolite profile explained by the top 12 species (B), 15 MetaCyc pathways (C), and 15 fecal metabolites (D). (E) A polar bar plot illustrates the extent of variance explained by gut microecological features for each serum metabolite. The figure includes only those metabolites with an explained variance greater than 1%. Abbreviations: 3S-GLCA: sulfated glycosylcholic acid; EPA: eicosapentaenoic acid; HPHPA: 3-(3-Hydroxyphenyl)-3- hydroxypropanoic acid.

adjusted HR in Fig. 3B and C), along with serum metabolites (citramalic acid, sebacic acid, oxoglutaric acid, and glycoursodeoxycholic acid), were positively correlated, whereas serum eicosapentaenoic acid (EPA), chenodeoxycholic acid, suberic acid, and indoleacetic acid were inversely associated with the incidence of cardiometabolic diseases (Fig. 4A–I). The associations of 32-SMS with the risk of MS (HR 1.66, $P < 2E\text{-}16$) and CAP (HR 1.18, $P = 1.29E\text{-}6$) remained significant even after further adjusting for BMI as a confounding factor for sensitivity analyses. Additionally, significant nonlinear associations were observed between serum metabolites of EPA (Fig. 4H), suberic acid (Fig. 4F), sebacic acid (Fig. 4D), and glycoursodeoxycholic acid (Fig. 4I) and CAP risk, as well as between citramalic acid and MS risk (Fig. 4A).

Associations of GMM-SMs, serum proteins and incident cardiometabolic disease

We conducted an untargeted serum proteomics analysis to augment and contextualize our findings from serum metabolite and cardiometabolic incidence studies. A Sankey diagram illustrated the interplay between GMM-SMs, serum proteins, and cardiometabolic incidence. Notably, the 32-SMS and specific serum proteins (apolipoprotein A-I [apoA-I], glutathione peroxidase 3 [GPX3], sex hormone-binding globulin [SHBG], and C-type lectin domain family 3 member B [CLEC3B]

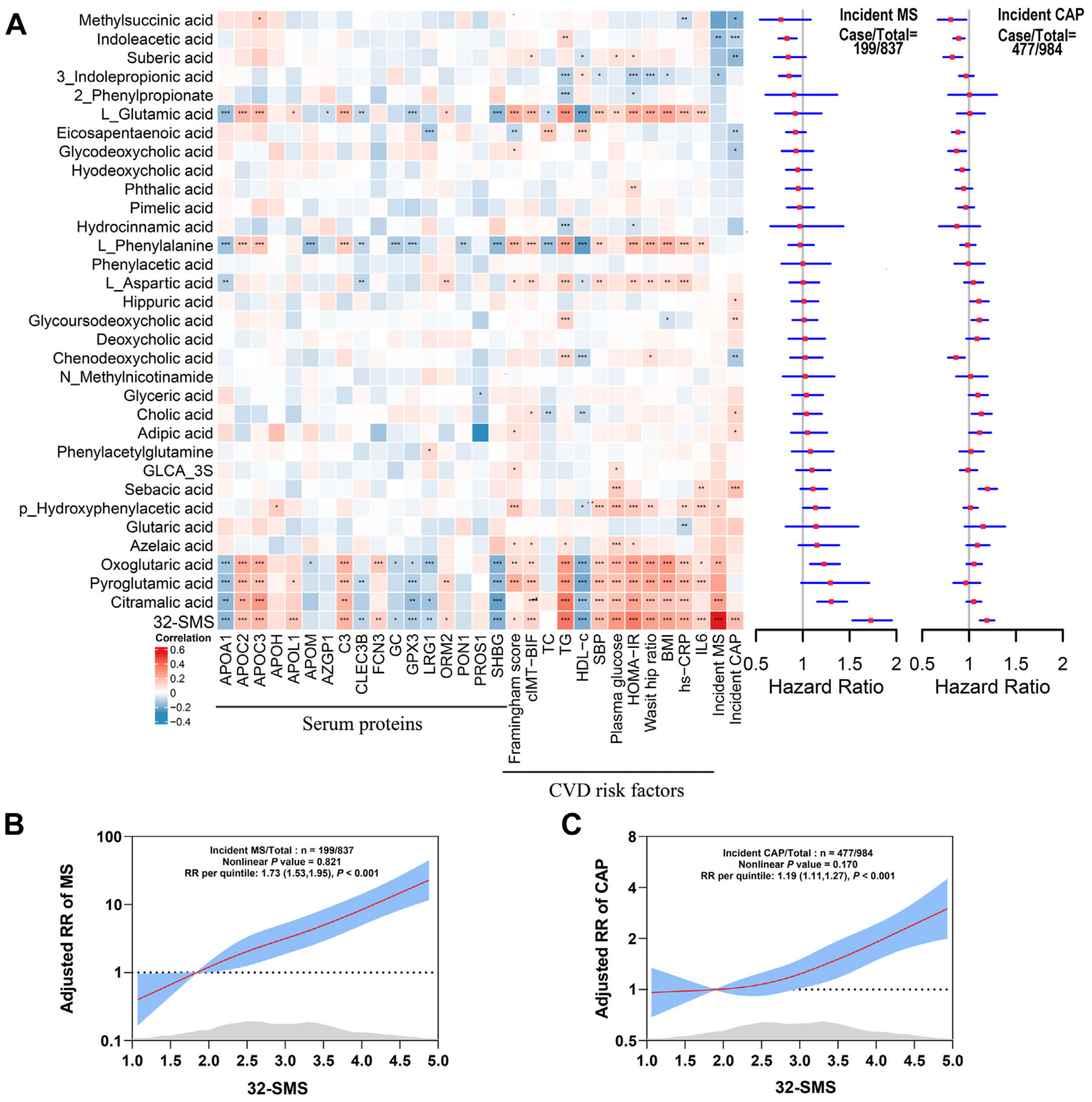

Fig. 3: **Associations of GMM-explained serum metabolites with serum proteins, CVD risk factors, and MS and CAP incidences.** (A) The heatmap shows the standardized beta coefficients between serum metabolites and proteins using linear regression and correlation coefficients between serum metabolites and CVD risk factors using Spearman correlation, alongside their corresponding *P* values. The forest plot presents the adjusted hazard ratios (HRs) and 95% confidential intervals (95% CI) of incident MS and CAP by each quintile increase in the top 32 GMM-explained serum metabolites and the 32-metabolite score (32-SMS), as analyzed by the Cox regression model. Significance levels are indicated as * *P* < 0.05, ***P* < 0.01, ****P* < 0.001. (B, C) Relative risks (RRs) and their 95% CIs of incident MS (B) and CAP (C) in relation to the 32-SMS. We analyzed using a linear model and restricted cubic spline method of Cox regression. The reference value is set at the median of the first quintile in the 32-SMS with nodes at the 20th, 40th, 60th, and 80th percentiles. The 32-SMS was developed using the β coefficients between the top 32 GMM-explained serum metabolites and MS incidence. Covariates adjusted: age, sex, education, household income, marital status, smokers, tea drinkers, alcohol drinkers, physical activity, multivitamin use, and daily energy intake. Abbreviations: APOA1: apolipoprotein A1; APOC2: apolipoprotein C2; APOC3: apolipoprotein C3; APOH: apolipoprotein H; APOL1: apolipoprotein L1; APOM: apolipoprotein M; AZGP1: alpha-2-glycoprotein 1, zinc-binding; BMI: body mass index; C3: complement protein 3; CAP: carotid atherosclerotic plaque; cIMT-BIF: carotid intima-media thickness at the bifurcation; CLEC3B: c-type lectin domain family 3 member B; CVD: cardiovascular disease; FCN3: Ficolin 3; GC: GC vitamin D binding protein; GLCA-3S: sulfated glycosylcholic acid; GMM: gut microbiota and metabolites; GPX3: glutathione peroxidase 3; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; hs-CRP: high sensitivity C-Reactive Protein; IL-6: interleukin-6; LRG1: leucine-rich alpha-2 glycoprrotein 1; MS: metabolic syndrome; ORM2: orosomucoid 2; PON1: paraoxonase and arylesterase 1; PROS1: protein S; RR: relative risk; SBP: systolic blood pressure; SHBG: sex hormone binding globulin; TC, total cholesterol; TG: triglycerides.

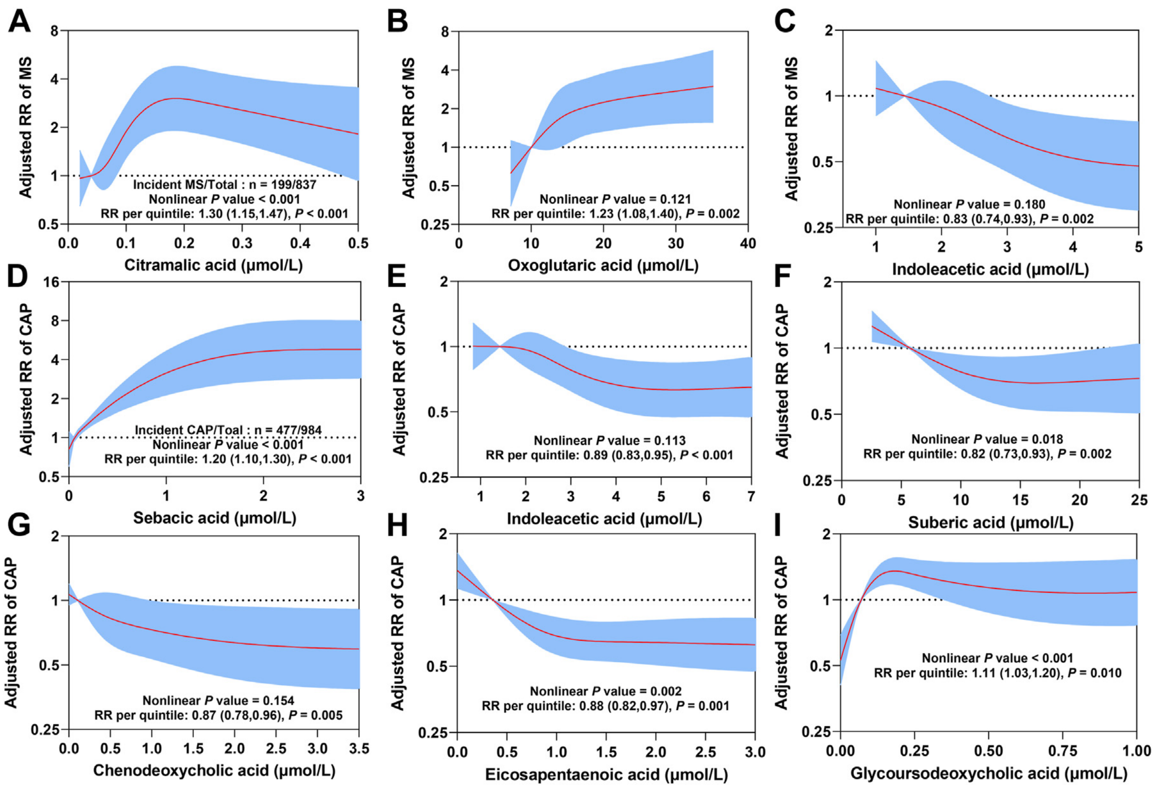

Fig. 4: **Associations of top GMM-explained serum metabolites and incident MS and CAP**. A-C, the adjusted relative risks (RRs) and 95% confidential intervals (95% CI) of incident metabolic syndrome (MS) associated with serum citramalic acid (A), oxoglutaric acid (B), and indoleacetic acid (C). D-I, Adjusted RRs and 95% CI of incident carotid atherosclerotic plaque (CAP) associated with serum sebacic acid (D), indoleacetic acid (E), suberic acid (F), chenodeoxycholic acid (G), eicosapentaenoic acid (H), and glycoursodeoxycholic acid (I), respectively. The restricted cubic spline method of Cox regression was used for the analysis. The reference point was set at the median of the first quintile in the serum metabolites with values above zero, with nodes positioned at the 20th, 40th, 60th, and 80th percentiles. Covariates adjusted in these analyses: age, sex, education, household income, marital status, smokers, tea drinkers, alcohol drinkers, physical activity, multivitamin use, daily energy intake, and other significant serum metabolites identified through step-wise variable selection. A forward selection process in the Cox model was utilized to determine the most predictive variables ($P < 0.05$) from the potential candidates. The Cox proportional-hazards model was employed to develop the model and estimate the coefficients for each predictor, indicating the RR per quintile. GMM: gut microbiota and metabolites.

showed inverse associations, and these proteins were also inversely associated with MS incidence. Conversely, 32-SMS was positively associated with orosomucoid 2 (ORM2), apoC-II, apoC-III, apoL-I, and complement protein 3 (C3), which in turn were positively linked to MS incidence (Fig. 5). Our data satisfied all linear regression model prerequisites: linear relationships, error term normality and homoscedasticity, and observation independence (Fig. S2). The *P*-value associated with a global test of proportionality in the Cox models were reported in Table S13, which suggested evidence of proportionality (all $P > 0.40$).

Network between GMM, SMs and cardiometabolic traits

A comprehensive network delineates the intricate connections between GMM, SMs, and cardiometabolic traits, with relationships quantified by Spearman correlation coefficients (Fig. 6). Central to this network are nodes including key SMs (glutamic acid, pyroglutamic acid, L-phenylalanine, citramatic acid, oxoglutaric acid, sebacic acid, suberic acid, and glycoursodeoxycholic acid). These nodes link GMM constituents to various cardiovascular risk factors. The GMM constituents included particularly microbial species from *Eubacterium*, *Clostridium*, and *Ruminococcus* genera, metabolic pathways (e.g., urea cycle [PWY4984], L-proline biosynthesis [PWY4981], and tetrapyrrole biosynthesis I [PWY5188]), and specific fecal metabolites (like fatty acids [sebacic acid and suberic acid], bile acids [ursodeoxycholic acid, nordeoxycholic acid, muro cholic acid, and glycoursodeoxycholic acid]) (Fig. 6).

Discussion

This study demonstrates that the combined GMM factors (species, MetaCyc pathways, and fecal metabolome) accounted for 15.1% of the variance in total SM profiles

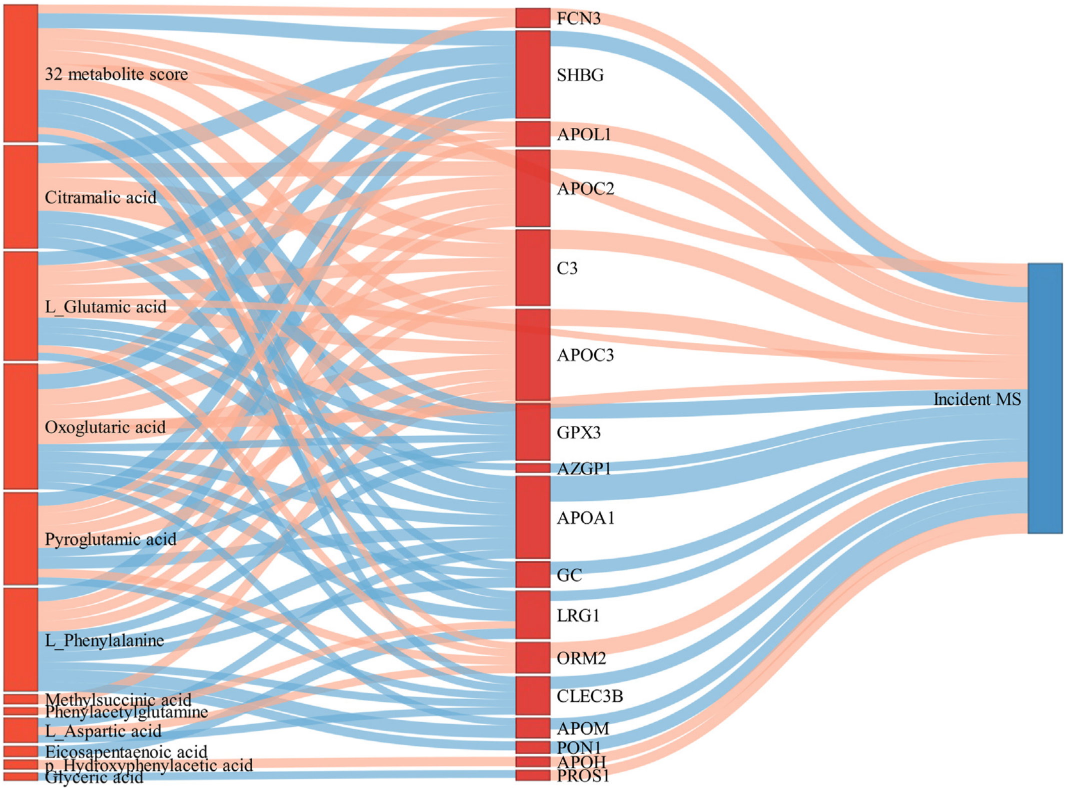

Fig. 5: **Interrelationships among GMM-explained serum metabolite indices, CVD-related proteins, and incident MS based on regression coefficients**. This illustrates the standardized β coefficients from linear regression associations between GMM-explained serum metabolites and serum proteins, as well as β coefficients between serum proteins (per quintile) and incident metabolic syndrome (MS) by Cox regression. We adjusted the *P*-values by using the Benjamini-Hochberg false discovery rate (FDR) method. Light pink and blue connections indicate positive and inverse correlations (FDR<0.05). Rectangles represent GMM-explained serum metabolite indices (left), serum proteins (middle), and incident MS (right). Abbreviations: APOA1: apolipoprotein A1; APOC2: apolipoprotein C2; APOC3: apolipoprotein C3; APOH: apolipoprotein H; APOL1: apolipoprotein L1; APOM: apolipoprotein M; AZGP1: alpha-2-glycoprotein 1, zinc-binding; C3: complement protein 3; CAP: carotid atherosclerotic plaque; CLEC3B: c-type lectin domain family 3 member B; CVD: cardiovascular disease; FCN3: Ficolin 3; GC: GC vitamin D binding protein; GMM: gut microbiota and metabolites; GPX3: glutathione peroxidase 3; LRG1: leucine-rich alpha-2 glycoprotein 1; MS: metabolic syndrome; ORM2: orosomucoid 2; PON1: paraoxonase and arylesterase 1; PROS1: protein S; SHBG: sex hormone binding globulin.

and a substantial 5.5%–49.6% in 32 individual SM profiles. Particularly noteworthy among these top 32 GMM-explained SMs, the 32-SMS, along with citramalic acid, oxoglutaric acid, EPA, indoleacetic acid, suberic acid, chenodeoxycholic acid, glycoursodeoxycholic acid, and sebacic acid emerged as key SMs linking GMM and the incidence of MS and CAP. The elucidation of these GMM-associated SMs, their related proteins, and their interplay with cardiometabolic health offers a solid foundation for identifying potential intervention targets and deepening our understanding of the role of gut microbiome in health modulation.

The contribution of GMM on serum metabolome

The interplay between gut microbiota and the host significantly influences SM composition and their concentrations. This study highlights the notable influence of GMM on the serum metabolome, explaining 15.1% of its variance. This is comparable to findings from two recent studies: one with 491 Israeli participants reporting a 30.8% influence,[6] and another with 1368 participants from the Netherlands showing a 12.8%–27.8% impact.[7] Notably, the primary SM categories influenced by GMM vary across populations. In the Israeli individuals, xenobiotics, unidentified metabolites, and lipids were predominantly affected,[6] while in the Dutch study, microbial abundances contributed 0.04%–25.1% variance in 208 of 1183 plasma metabolites, with p-cresol, sulfurous acid, p-cresol sulfate, acetyl-N-formyl-5-methoxykynurenamine, and 2,3-diketogulonate being the most impacted.[7]

Our analysis of 168 SMs revealed that over 40% of the variance in 3S-GLCA (bile acids), 2-phenylpropionic acid, and hydrocinnamic acid (phenylpropanoic acids) could be attributed to microecological factors. This finding positions our study as the first to identify SM signatures significantly influenced by GMM. However,

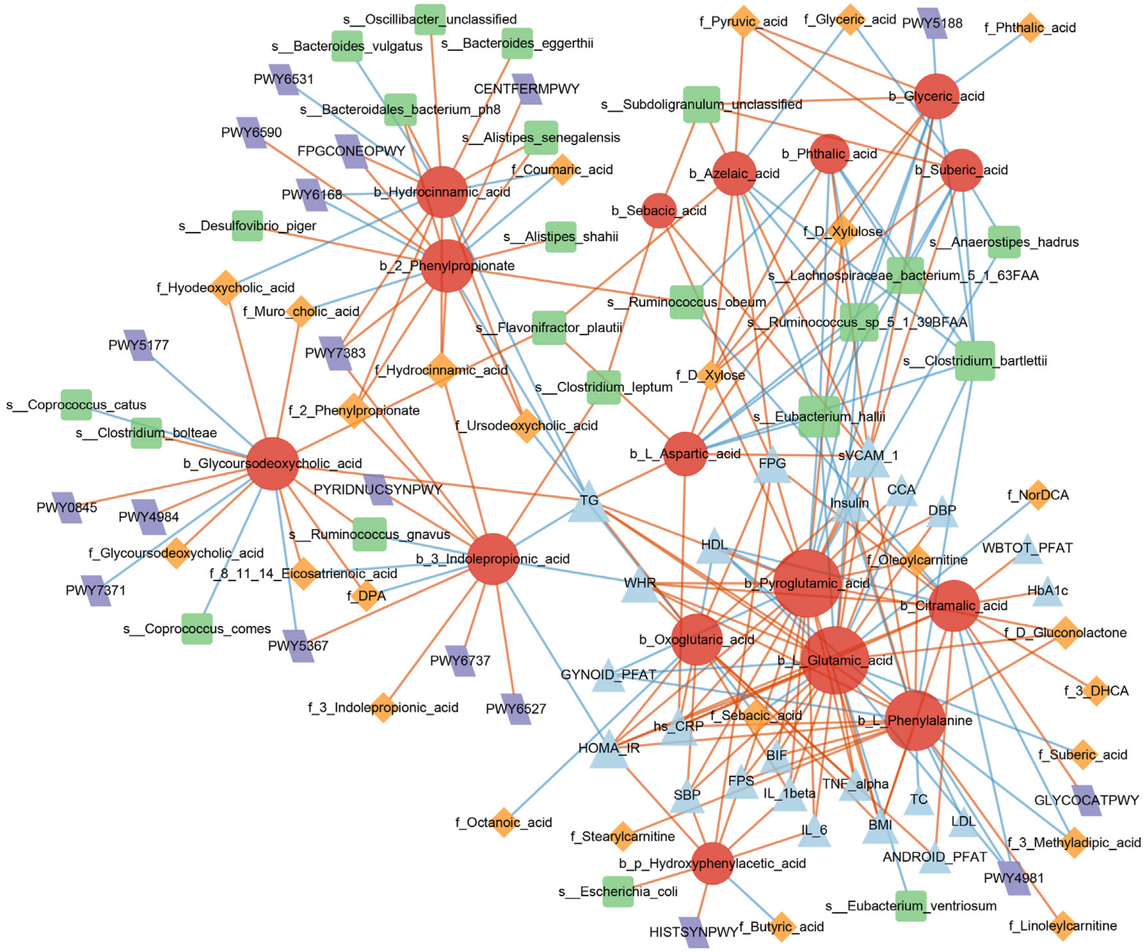

Fig. 6: **Network illustrating relationships between gut microecological signatures, serum metabolites and cardiometabolic traits**. The network diagram visualizes the relationships between gut microecological signatures, serum metabolites, and cardiometabolic traits, constructed based on Spearman coefficients exceeding 0.12 (all $P < 0.00015$). Each dot represents a distinct feature, with the size indicating the number of connections. The color of the lines, blue for inverse and red for positive correlations, along with their thickness, represents the strength of the associations. Key elements are color-coded for easy identification: green round rectangles for species, purple parallelograms for MetaCyc pathways, orange diamonds for fecal metabolites, red ellipses for blood metabolites, and blue triangles for cardiometabolic traits. Abbreviations: ANDROID_PFAT, fat mass percentage at the android region; BIF, carotid intima-media thickness of the bifurcation; CCA, carotid intima-media thickness of the common carotid artery; FPS, Framingham point score; GLU, fasting blood glucose; GYNOID_PFAT, fat mass percentage at the gynoid region; HbA1c, glycated hemoglobin; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; hs-CRP, high sensitivity C-Reactive Protein; IL-1β, interleukin-1β; IL-6, interleukin-6; LDL-c, low-density lipoprotein cholesterol; NorDCA, nordeoxycholic acid; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-α; WB_PFAT, fat mass percentage at the whole body. s_: species; f_: fecal metabolites; b_: blood metabolites.

given the limited evidence and substantial between-population heterogeneity, further research is essential to elucidate the global influence of GMM on the serum metabolome.

Microecological contributors for SMs

Unraveling the microecological contributors to SM profiles is pivotal for pinpointing target microbiota for health intervention and understanding microbiome–health interactions. This research marks a pioneering effort in delineating the key features of microbial species, pathways, and fecal metabolites correlating with SMs in an Asian population. A significant finding is the role of *Eubacterium hallii* in shaping the microbiota-SM relationship. In terms of serum bile acids, *Clostridium* genus and fecal lithocholic acid emerged as the primary predictors for the most explained two bile acids (serum 3S-GLCA and glycodeoxycholic acid). Intriguingly, both *Eubacterium hallii* and genus *Clostridium* belong to the *Firmicutes* phylum, identified as the principal bacterial influence on blood metabolites in Bar et al.' study.[6] Lithocholic acid, a microbiota-derived monohydroxy bile acid[22] and primarily existing in the form of a conjugate called GLCA,[23] underscores the microbiome's significant role in metabolite modulation. These insights offer valuable targets within GMM for potential interventions aimed at altering SM profiles.

Role of GMM-related SMs in cardiometabolic health

This study underscores the significant correlations between certain GMM-related SMs and cardiometabolic disease incidence. Specifically, serum citramalic acid and sebacic acid levels exhibited positive correlations, while serum EPA, chenodeoxycholic acid, and indoleacetic acid levels were inversely associated with MS or CAP incidence. Citramalic acid, known to be produced by microbiota,[24] and sebacic acid, a key intermediate in the tricarboxylic acid cycle, have been linked to cardiovascular health. Notably, sebacic acid levels were elevated in atherosclerotic rat models.[25] EPA, a long-chain omega-3 polyunsaturated acid, is renowned for its cardioprotective properties,[26–28] including plasma triglyceride reduction,[27] reducing proinflammatory cytokines,[29] and inhibiting plaque progression in those with coronary heart disease.[29] Chenodeoxycholic acid, an endogenous metabolite, is implicated in anti-tumor activity via the Epidermal Growth Factor Receptor/Stat3 pathway[30] and correlates positively with lean body mass levels.[31] Indolepropionic acid can catalyze the synthesis of indoleacetic acid.[32] Dietary indolepropionic acid supplementation attenuates atherosclerotic plaques.[33] These collectively highlight the potential of GMM-explained SMs (e.g., sebacic acid, EPA, and indoleacetic acid) in modulating cardiometabolic disease risk.

Interplay of SMs, proteins, and cardiometabolic disease incidence

This study sheds light on the intricate relationship between SMs, closely linked to GMM, and serum proteins related to CVD, offering insights for future interventions targeting specific proteins to mitigate CVD risk. Among GMM-SM-related serum proteins, apoA-I exhibited a negative association with MS incidence. Notably, fat-free apoA-I interacts with the adenosine 5'triphosphate-binding cassette transporter A1, aiding in the creation of nascent HDL particles in the gut.[33,34] In contrast, GMM-SM-related apoC-II, apoC-III, and C3 levels showed positive association with incident MS in this population. ApoC, synthesized in the gut,[35] plays a crucial role in lipid metabolism. Elevated apoC-II levels are associated with an increase in triglyceride-rich particles and changes in HDL particle distribution, potentially heightening CVD risk.[36] ApoC-III contributes to atherosclerosis progression by facilitating the retention and accumulation of LDL in the subendothelial space, and by stimulating inflammatory cascades and the proliferation of smooth muscle cells.[36,37] In addition, C3, a crucial complement, is implicated in various metabolic disorders, particularly obesity, dyslipidemia, insulin resistance, and diabetes.[38] Taken together, these results indicate that the notable correlations between GMM-SMs and cardiometabolic diseases may be partly ascribed to the modulation of specific apolipoproteins and C3.

Potential mechanisms linking GMM, SMs, proteins and cardiometabolic health

Our study revealed an inverse association of the species *Ruminococcus_obeum* and fecal 3-Methyladipic acid with serum citramalic acid. Intriguingly, citramalic acid, linked to C3, which was positively correlated to incident MS. Both 3-methyladipic acid and citramalic acid, classified as organic acids,[39] have distinctive biosynthesis pathways and health implications. Prior research has demonstrated that citramalic acid can be synthesized by the microbiota.[24] In another study, citramalic acid was higher in obese women.[40] Increasing evidence highlights the ability of C3 that might lead to MS. C3adesArg, also referred to as the acylation stimulation factor, augments the synthesis and discharge of triglycerides in adipocytes. These physiological impacts substantially contribute to the emergence of MS.[41]

Strengths

This study stands out with several notable strengths. Primarily, to our knowledge, it is the first to delineate the signatures of GMM-related SMs and to investigate their association with serum proteins and the risk of incident MS and CAP, utilizing a comprehensive approach that includes both microecological and host multi-omics. Another significant advantage was the use of metagenomics for microbiota analysis, providing a more detailed representation compared to the 16S rRNA method. Additionally, the additional analysis of the fecal metabolome substantially improved our understanding and explanation of SMs beyond what metagenomic analysis alone could offer. Furthermore, the substantial size of the study bolstered the statistical power necessary to detect meaningful associations between the variables. This robust study design has enabled a deeper exploration of the complex interplay between fecal microbiome, SMs, and cardio-metabolic health, thereby contributing valuable insights to this field of research.

Caveats and limitations

Despite its strengths, this study is subject to several caveats and limitations. Firstly, the nature of the observational study precludes the establishment of causality in the observed associations, necessitating validation through further experimental studies. Given the impracticality of long-term germ-free humans and the substantial differences in intestinal microecology between humans and animals, our research offers valuable evidence for potential interventions and understanding of microecological features in cardiometabolic health. Secondly, the recruitment of participants from a single city might impose limitations on the generalizability of our findings to other ethnicities or regions, especially considering the significant microbiota variations across different genetic and environmental backgrounds.[38] Thirdly, the need for replication cohort studies is evident to reinforce our findings and minimize the possibility of chance results. While our study presents a significant contribution to the existing scientific knowledge, corroborating these findings in diverse populations is essential. Lastly, a longitudinal omics analysis over an extended period and at regular intervals would yield deeper insights into the onset and progression of cardiometabolic diseases. Our choice to use mid-term samples from our cohort was driven by the substantial advancements in multi-omics techniques over recent years, and the pivotal role of mid-term GMM and SMs status in understanding the development of MS and CAP.

Conclusions and implications

This study reveals the significant influence of GMM on SM profiles, particularly identifying key serum metabolites linked to MS and CAP incidence. These findings offer valuable insights into potential intervention targets and enhance our understanding of the role of microbiome in health via SMs. The complex relationships between gut microecology, metabolomics, proteomics, and cardiometabolic health could lead to personalized treatment strategies for these prevalent diseases. This knowledge is especially pertinent for developing novel therapeutic interventions, particularly for managing complex health conditions in the elderly.

Contributors

Y.-m.C., J.-S.Z., and X.-y.T. contributed to the conceptualization and design of the study. C.-w. L. contributed to data analysis. C.-w. L., K. D, Y. Y., H.-r.L., C.-y. L., B.-y. L., W.H., X.L., and H.Z. contributed to data collection. C.-w. L. contributed to the manuscript writing. C.-w. L. and Y.-m.C. have directly accessed and verified the underlying data reported in the manuscript. Y.-m.C. and J.-S.Z. wrote, reviewed, and edited the manuscript. Y.-m.C. and J.-S.Z. are the guarantors of this work and take responsibility for the integrity of the data and accuracy of the data analysis. Y.-m.C. was responsible for the decision to submit the manuscript. All authors read, revised, and approved the final draft of the manuscript and confirmed that they had full access to all the data in the study and accept responsibility to submit for publication. C.-w. L., K.D., Y.Y., and H.-r.L. contributed equally to the work. X.-y.T., J.-S.Z., and Y.-m.C. contributed equally to the work.

Data sharing statement

Raw metagenomic sequencing data are available at the CNSA (https://db.cngb.org/cnsa/) of CNGBdb (accession number CNP0001510). Other data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105209.

References

1 *WHO. Cardiovascular diseases (CVDs)*; 2021. Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).

2 Cannon CP. Cardiovascular disease and modifiable cardiometabolic risk factors. *Clin Cornerstone*. 2007;8(3):11–28.

3 Hansen TH, Gobel RJ, Hansen T, Pedersen O. The gut microbiome in cardio-metabolic health. *Genome Med*. 2015;7(1):33.

4 de Vos WM, Tilg H, Van Hul M, Cani PD. Gut microbiome and health: mechanistic insights. *Gut*. 2022;71(5):1020–1032.

5 Wang Z, Zhao Y. Gut microbiota derived metabolites in cardiovascular health and disease. *Protein Cell*. 2018;9(5):416–431.

6 Bar N, Korem T, Weissbrod O, et al. A reference map of potential determinants for the human serum metabolome. *Nature*. 2020;588(7836):135–140.

7 Chen L, Zhernakova DV, Kurilshikov A, et al. Influence of the microbiome, diet and genetics on inter-individual variation in the human plasma metabolome. *Nat Med*. 2022;28(11):2333–2343.

8 Zierer J, Jackson MA, Kastenmuller G, et al. The fecal metabolome as a functional readout of the gut microbiome. *Nat Genet*. 2018;50(6):790–795.

9 Deng K, Xu JJ, Shen L, et al. Comparison of fecal and blood metabolome reveals inconsistent associations of the gut microbiota with cardiometabolic diseases. *Nat Commun*. 2023;14(1):571.

10 Marcobal A, Kashyap PC, Nelson TA, et al. A metabolomic view of how the human gut microbiota impacts the host metabolome using humanized and gnotobiotic mice. *ISME J*. 2013;7(10):1933–1943.

11 Ling CW, Zhong H, Zeng FF, et al. Cohort profile: Guangzhou nutrition and health study (GNHS): a population-based multi-omics study. *J Epidemiol*. 2023;34:301.

12 Truong DT, Franzosa EA, Tickle TL, et al. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. *Nat Methods*. 2015;12(10):902–903.

13 Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81(3):559–575.

14 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):1564–1573.

15 Zhu CH, Wei Y, Chen F, et al. Investigation on the mechanisms of human sperm DNA damage based on the proteomics analysis by SWATH-MS. *Clin Proteomics*. 2023;20(1):2.

16 Rost HL, Rosenberger G, Navarro P, et al. OpenSWATH enables automated, targeted analysis of data-independent acquisition MS data. *Nat Biotechnol*. 2014;32(3):219–223.

17 Zhu T, Zhu Y, Xuan Y, et al. DPHL: a DIA pan-human protein mass spectrometry library for robust biomarker discovery. *Dev Reprod Biol*. 2020;18(2):104–119.

18 Yang Q, Cogswell ME, Flanders WD, et al. Trends in cardiovascular health metrics and associations with all-cause and CVD mortality among US adults. *JAMA*. 2012;307(12):1273–1283.

19 Touboul PJ, Hennerici MG, Meairs S, et al. Mannheim intima-media thickness consensus. *Cerebrovasc Dis*. 2004;18(4):346–349.

20 Dixon P. VEGAN, a package of R functions for community ecology. *J Veg Sci*. 2003;14(6):927–930.

21 Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498–2504.

22 Sheng W, Ji G, Zhang L. The effect of lithocholic acid on the gut-liver Axis. *Front Pharmacol*. 2022;13:910493.

23 Bansal S, Lau AJ. Inhibition of human sulfotransferase 2A1-catalyzed sulfonation of lithocholic acid, glycolithocholic acid, and taurolithocholic acid by selective estrogen receptor modulators and various analogs and metabolites. *J Pharmacol Exp Ther*. 2019;369(3):389–405.

24 Wu X, Tovilla-Coutino DB, Eiteman MA. Engineered citrate synthase improves citramalic acid generation in Escherichia coli. *Biotechnol Bioeng*. 2020;117(9):2781–2790.

25 Jia P, Wang S, Xiao C, et al. The anti-atherosclerotic effect of tanshinol borneol ester using fecal metabolomics based on liquid chromatography-mass spectrometry. *Analyst*. 2016;141(3):1112–1120.

26 Matsumoto M, Sata M, Fukuda D, et al. Orally administered eicosapentaenoic acid reduces and stabilizes atherosclerotic lesions in ApoE-deficient mice. *Atherosclerosis*. 2008;197(2):524–533.

27 Nakajima K, Yamashita T, Kita T, et al. Orally administered eicosapentaenoic acid induces rapid regression of atherosclerosis via modulating the phenotype of dendritic cells in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol*. 2011;31(9):1963–1972.

28 Motoyama S, Nagahara Y, Sarai M, et al. Effect of omega-3 fatty acids on coronary plaque morphology–a serial computed tomography angiography study. *Circ J*. 2022;86(5):831–842.

29 Li H, Ruan XZ, Powis SH, et al. EPA and DHA reduce LPS-induced inflammation responses in HK-2 cells: evidence for a PPAR-gamma-dependent mechanism. *Kidney Int*. 2005;67(3):867–874.

30 Zhang Y, Zhang Y, Shi XJ, et al. Chenodeoxycholic acid enhances the effect of sorafenib in inhibiting HepG2 cell Growth through EGFR/Stat 3 pathway. *Front Oncol*. 2022;12:836333.

31 Zhang YH, Wang WY, Cui YM. Gut microbiome metabolism drives the resolution of patients with coronavirus disease 2019. *Gastroenterology*. 2022;163(1):336–337.

32 Wu X, Liu K, Wu Q, et al. Biomarkers of metabolomics in inflammatory bowel disease and damp-heat syndrome: a preliminary study. *Evid Based Complement Alternat Med*. 2022;2022:3319646.

33 Xue H, Chen X, Yu C, et al. Gut microbially produced indole-3-propionic acid inhibits atherosclerosis by promoting reverse cholesterol transport and its deficiency is causally related to atherosclerotic cardiovascular disease. *Circ Res*. 2022;131(5):404–420.

34 Gillotte KL, Zaiou M, Lund-Katz S, et al. Apolipoprotein-mediated plasma membrane microsolubilization. Role of lipid affinity and membrane penetration in the efflux of cellular cholesterol and phospholipid. *J Biol Chem*. 1999;274(4):2021–2028.

35 Ooi EM, Barrett PH, Chan DC, Watts GF. Apolipoprotein C-III: understanding an emerging cardiovascular risk factor. *Clin Sci*. 2008;114(10):611–624.

36 Kei AA, Filippatos TD, Tsimihodimos V, Elisaf MS. A review of the role of apolipoprotein C-II in lipoprotein metabolism and cardiovascular disease. *Metabolism*. 2012;61(7):906–921.

37 Kawakami A, Aikawa M, Nitta N, Yoshida M, Libby P, Sacks FM. Apolipoprotein CIII-induced THP-1 cell adhesion to endothelial cells involves pertussis toxin-sensitive G protein- and protein kinase C alpha-mediated nuclear factor-kappaB activation. *Arterioscler Thromb Vasc Biol*. 2007;27(1):219–225.

38 Hertle E, van Greevenbroek MM, Stehouwer CD. Complement C3: an emerging risk factor in cardiometabolic disease. *Diabetologia*. 2012;55(4):881–884.

39 Kim KR, Park HG, Paik MJ, et al. Gas chromatographic profiling and pattern recognition analysis of urinary organic acids from uterine myoma patients and cervical cancer patients. *J Chromatogr B Biomed Sci Appl*. 1998;712(1–2):11–22.

40 Escobar-Morreale HF, Samino S, Insenser M, et al. Metabolic heterogeneity in polycystic ovary syndrome is determined by obesity: plasma metabolomic approach using GC-MS. *Clin Chem*. 2012;58(6):999–1009.

41 Copenhaver M, Yu CY, Hoffman RP. Complement components, C3 and C4, and the metabolic syndrome. *Curr Diabetes Rev*. 2019;15(1):44–48.