Genetically predicted 1,400 blood metabolites related to the risk of ovarian cancer: A Mendelian randomization study

Keywords
ovarian cancer, Mendelian randomization, genomic metabolites, MR-Egger, Cochrane Q

Abstract

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Material and methods

This study employed a GWAS and applied a two-sample MR analysis. Causality was primarily assessed using random IVW. Cross-validation was conducted with MR-Egger, weighted median, and weighted mode approaches. The MR-Egger intercept and Cochran’s Q test were adopted to assess heterogeneity and pleiotropy. Pathway enrichment analysis was performed using MetaboAnalyst 6.0.

Results

Our research identifies key metabolites as potential biomarkers for early screening and personalized therapy in ovarian cancer. By using MR, we establish causal links between ovarian cancer subtypes and plasma metabolites, offering valuable insights for clinical applications. After FDR correction, screening for one metabolite, 5-acetylamino-6-amino-3-methyluracil levels (AAMU). Also, significant metabolites were enriched to caffeine metabolism($p<0.05$) as the most significant metabolic pathway in ovarian cancer.

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We integrated genomic and metabolomic analyses to reveal causal associations of metabolites with ovarian cancer and its subtypes. Certain metabolites were indicated as prospective biomarkers for ovarian cancer

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Introduction:

OC ranks among the three most prevalent cancers affecting the female reproductive system(1). In the year 2020, 313,959 new OC cases have been diagnosed worldwide, equivalent to 6.6 ASR cases per 100,000 people. The regions with the highest reported incidence rates include Central and Eastern Europe, where the ASR is notably elevated at 10.7. Northern Europe, which has an ASR of 8.8, follows closely(2). Currently, several molecular biomarkers, such as CA125 and HE4, are utilized for ovarian cancer screening and monitoring. However, their sensitivity, specificity, and applicability have certain limitations, and their performance in early OC screening remains suboptimal. Because early diagnostic indicators and symptoms are lacking, most OC patients are

diagnosed at advanced stages(3, 4). Current molecular strategies to prevent ovarian cancer include the use of oral contraceptives to reduce OC risk, the role of genetic testing (BRCA1/2 mutations), and ongoing research on targeted therapies aimed at preventing the progression of high-risk ovarian lesions. But all have significant limitations. In addition, OC is a multifaceted process that involves many variables, which creates an enormous public health problem.

According to previous studies, the development of OC is associated with genetic factors(5), estrogen levels(6), age(7), abnormal gene splicing(8) and metabolic disorders(9), which are considered important contributors, and the progression of OC may be due to combined effects. Metabolic disorders are important factors in the development and progression of many diseases(10-12). Metabolic disorders, as one of the hallmark features of cancer, also is the key to OC; for example, Zhou(13) reported significant differences in the metabolites of histamine, purine nucleotides, glycine, serine, and sarcosine, along with the overexpression of alanine, cysteine, and glycine in serum samples by mass spectrometry in 44 ovarian serous cystadenocarcinoma (Stages I--IV) samples and in 50 healthy females or those with benign disease. Many plasma metabolites of small metabolic molecules are known to play key intermediate functions in different physiological pathways(14, 15). Their metabolism provides valuable insights into underlying pathological conditions, including cancer, which make them excellent candidates for biomarker exploration. However, evidence for a causal role of this trait in promoting or preventing OC is still lacking. Therefore, the utilization of plasma metabolites as potential biomarkers for early OC detection and as viable targets for innovative therapeutic interventions to enhance cancer prevention and early screening is a critical priority strategy.

MR is a significant tool in epidemiology, as it addresses confounding variables and uncovers possible causal links. Research indicates that genetic variations can affect biochemical concentrations in plasma, implying that genetic diversity might contribute to racial disparities in the variations related to sex and/or age at the metabolite level(16, 17). A recent thorough investigation into metabolite GWASs revealed loci connected to various diseases(18). In addition, Yiheng Che developed a GDM database to categorize genotype-dependent metabolic traits. This resource links thousands of metabolites and metabolic pathways to genetic data, enabling deeper exploration of the hidden connections between human plasma metabolites and the development and progression of OC(19).

This research identifies key metabolic signatures that contribute to OC progression. By using Mendelian Randomization, we establish causal links between OC subtypes and plasma metabolites, offering potential and easily detectable biomarkers for early detection. Our findings also suggest the possibility of integrating metabolomic profiling into clinical practice for improved diagnosis, screening, and personalized treatment strategies, ultimately advancing targeted therapies and prognostic tools for OC.

METHODS

2.1 Study design

This study utilized the MR method in a large-scale GWAS to explore the fundamental relationships between the metabolites and OC, including its different subtypes. This approach is founded on three key assumptions(20, 21). This methodology depends on three essential assumptions: (1) The selected IVs must show a strong correlation with the exposure, specifically OC. (2) The selected IVs must be independent of any confounders that could affect the exposure–outcome relationship. (3) The selected IVs must influence the results solely through the exposure–outcome relationship. All analyses were conducted via R software (version 4.2.1) with the two-sample MR, MRCAMO, and radial MR packages. **Fig.1** provides the MR study process. This study investigated the causal associations between 1,400 plasma metabolites and OC risk, including its subtypes.

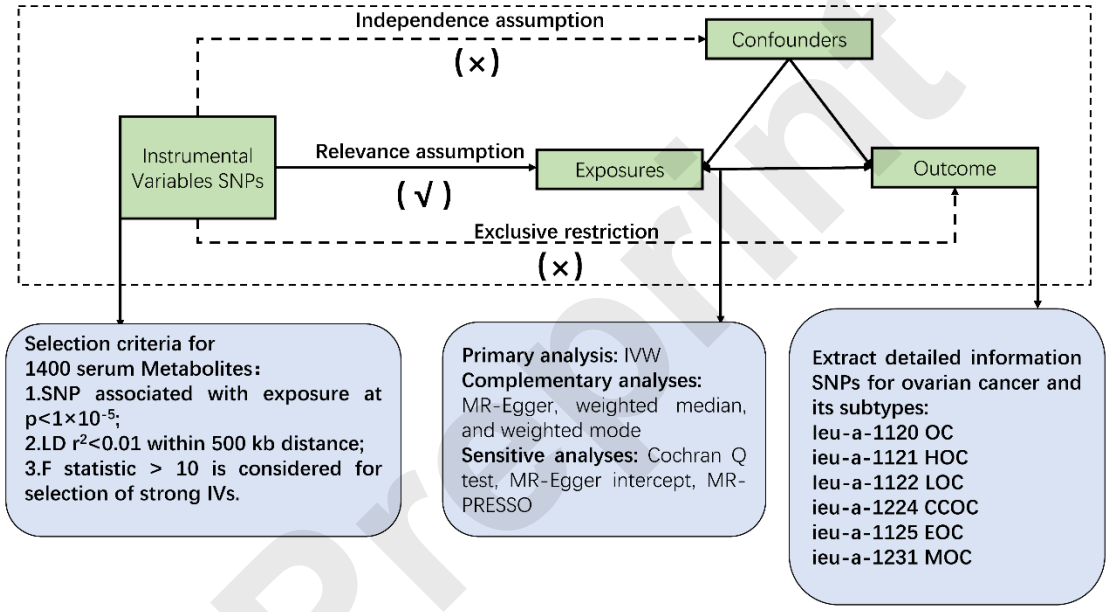

Fig. 1 The flowchart illustrates the complete workflow of the MR analysis.

2.2 Data sources for exposure and outcome

The plasma metabolites of MR were obtained from a study by Chen(19) involving approximately 8,000 individuals of European. A list of the summary indicators of GWASs focusing on plasma metabolites is available in the GWAS Catalog. The accession numbers for these GWAS data range from GCST90199621 to GCST90201020. MR data of OC and each subtype of OC were obtained from IEU database, OC dataset ID: ieu-a-1120. The analysis included 66,450 Europeans, of whom 225,509 had OC and 440,941 did not have OC. The data pertaining to the OC subtypes were derived from the same sources as previously described(22)(**Table 1**).

Table 1 The sources from which the data were obtained.

GWAS ID	Year	Trait	sample size	Ncase
ieu-a-1120	2017	OV	66450	25509
ieu-a-1121	2017	HOC	53978	13037
ieu-a-1122	2017	LOC	41953	1012
ieu-a-1124	2017	CCOC	42307	1366
ieu-a-1125	2017	EOC	43751	2801
ieu-a-1231	2017	MOC	43507	2566

Note: OC is currently classified into five major subtypes based on histopathologic and molecular genetic alterations: HOC, LOC, CCOC, EOC, MOC(22, 23).

2.3 Selection of instrumental variables

We accessed GWAS databases to identify IVs while adhering to the three main assumptions of MR. By employing a detailed selection methodology, we addressed concerns related to linkage disequilibrium and examined the latent causal relationship between plasma metabolites and OC. Recognizing the inherent nonindependence among metabolites, we acknowledge that adhering strictly to the conventional genome-wide significance threshold of $p<5\times10\text{-}8$ could be excessively cautious(19). Such an approach may inadvertently exclude potentially significant associations that warrant consideration. To broaden the scope of relevant plasma metabolites in our search, we applied a wider significance threshold of $P < 1\times10^{-5}$ for the selection of IVs(24). The intensity of the chosen SNPs as instruments was assessed by calculating the F statistic and the explained variance (R^2) for each IV relative to the exposure trait. A common threshold for strong IVs is F>10(25), and any IVs with an F<10 were excluded as weak instruments.

2.4 Statistical analysis

To assess latent heterogeneity in the Wald ratio estimates of SNPs(26), we applied the IVW method with multiplicative random effects for accurate estimation(27). If there is heterogeneity ($p < 0.05$), random effects IVW will be apply; If P>0.05, fixed-effects IVW was applied(28). In addition to IVW, three other MR methods—MR-Egger, weighted median, and weighted mode—were adopted to evaluate causality(29). The IVW approach supposes that all included SNPs are effective instruments(30). In contrast, the weighted median method requires that at least half of the genetic variants be valid and satisfy the core MR assumptions, making it particularly useful when most instruments are not influenced by horizontal pleiotropy(31). The MR-Egger regression method, however, posits that more than 50% of the genetic variants are invalid(32). Results from the four analyses were considered consistent only if the directions of the estimates (positive or negative) aligned(32-34). Should there be discrepancies in these directions, we ruled out any indirect causal associations between metabolites and the progression of OC.

We conducted two sensitivity analyses, Cochran's Q test and the MR-Egger intercept test to evaluate heterogeneity and pleiotropy(31, 32, 35). Heterogeneity was examined through IVW and MR-Egger regression, and the Cochran's Q statistic was

utilized as the primary measure. A P value >0.05 for both IVW and MR-Egger indicated no significant heterogeneity(36, 37). Pleiotropy was assessed by the MR-Egger regression intercept, where a P value above 0.05 suggested no evidence of pleiotropy. Additionally, MR-PRESSO analysis was conducted to detect and exclude significant outliers. To minimize false-positive outcomes from multiple testing, we employed FDR correction to adjust for statistical bias in multiple comparisons.

2.5 Metabolic pathway enrichment analysis

Pathway enrichment was carried out with the help of MetaboAnalyst version 6.0. This analysis encompassed a thorough investigation of metabolic pathways through RaMP, which stands for the Relational Database of Metabolomic Pathways. RaMP integrates various biological pathways drawn from multiple reputable sources, including KEGG, Reactome, WikiPathways, and the Human Metabolome Data Bank (HMDB)(38). This investigation included only biological pathways previously connected to OC identified by IVW (PIVW< 0.01). Additionally, only metabolites that have been previously linked to OC and its subtypes through IVW (PIVW< 0.01) were considered for this study.

Results

Plasma metabolites causally responsible for OC

We performed MR analysis of 1400 blood metabolites and OC while excluding the inconsistency of results in the estimation direction (either positive or negative) in four methods. Although repeated trials were adjusted by the FDR method, we identified only one metabolite at the 0.05 significance level: AAMU levels: OR= 1.116, 95% CI: 1.060--1.174, PFDR<0.038.

Nevertheless, we detected 14 metabolites associated with OC at the p<0.01 significance level. These included six metabolites suggestive of a high risk of association with OC: AAMU levels with OR= 1.116, 95% CI: 1.060–1.174, PFDR<0.038; Ceramide (d18:1/14:0, d16:1/16:0) levels with OR= 1.122, 95% CI: 1.055–1.194, P=0.0003; and SM(d18:1/16:0(OH)) levels with OR= 1.096, 95% CI: 1.036–1.1160, P=0.0014; X-12221 levels with OR= 1.130, 95% CI: 1.050–1.217, P=0.0012; X-12410 levels with OR=1.114, 95% CI: 1.045–1.187, p=0.0010; and AFAMU levels with OR= 1.071, 95% CI: 1.031–1.113, P=0.0004. 8 Low-risk metabolites: m5U levels with OR= 0.938, 95% CI: 0.898--0.980, P=0.0046; 2R,3R-DHB levels with OR= 0.905, 95% CI: 0.857--0.955, P=0.0003; LAG (18:2/20:4) levels with OR= 0.928, 95% CI: 0.878--0.981, P=0.0083; Gamma-glutamyl-alpha-lysine levels with OR= 0.902, 95% CI: 0.844--0.965, P=0.0026; Linolenoylcarnitine (C18:3) levels with OR= 0.876, 95% CI: 0.810--0.947, P=0.0008; N-lactoyl phenylalanine levels with OR= 0.849, 95% CI: 0.769--0.937, P=0.0012; X-23678 levels with OR= 0.906, 95% CI: 0.842-0.974, P=0.0079; Spermidine to N-acetylputrescine ratio with OR= 0.893, 95% CI: 0.841-0.947, P=0.0002(**Fig.2**, Supplementary S1). The p value results of the four MR analyses for all positive results are shown in **Fig.3**.

No signs of pleiotropy or heterogeneity were found in the strong causative factors

mentioned earlier, indicating that the main results from the IVW approach in our research could support causal associations with minimal heterogeneity. The findings related to heterogeneity and horizontal pleiotropy are summarized in **Supplementary Table S2-S3.**

Exposure	No.of SNP	Method	OR(95% CI)	P
5−acetylamino−6−amino−3−methyluracil levels	34	Inverse variance weighted	1.12 (1.06 to 1.17)	0.000
5−methyluridine (ribothymidine) levels	25	Inverse variance weighted	0.94 (0.90 to 0.98)	0.005
2R,3R−dihydroxybutyrate levels	30	Inverse variance weighted	0.90 (0.86 to 0.96)	0.000
Linoleoyl−arachidonoyl−glycerol (18:2/20:4) [1] levels	19	Inverse variance weighted	0.93 (0.88 to 0.98)	0.008
Gamma−glutamyl−alpha−lysine levels	31	Inverse variance weighted	0.90 (0.84 to 0.96)	0.003
Ceramide (d18:1/14:0, d16:1/16:0) levels	26	Inverse variance weighted	1.12 (1.05 to 1.19)	0.000
Linolenoylcarnitine (C18:3) levels	21	Inverse variance weighted	0.88 (0.81 to 0.95)	0.001
N−lactoyl phenylalanine levels	16	Inverse variance weighted	0.85 (0.77 to 0.94)	0.001
Hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH)) levels	31	Inverse variance weighted	1.10 (1.04 to 1.16)	0.001
X−12221 levels	23	Inverse variance weighted	1.13 (1.05 to 1.22)	0.001
X−12410 levels	25	Inverse variance weighted	1.11 (1.04 to 1.19)	0.001
X−23678 levels	19	Inverse variance weighted	0.91 (0.84 to 0.97)	0.008
5−acetylamino−6−formylamino−3−methyluracil levels	22	Inverse variance weighted	1.07 (1.03 to 1.11)	0.000
Spermidine to N−acetylputrescine ratio	20	Inverse variance weighted	0.89 (0.84 to 0.95)	0.000

Figure 2 Forest plot depicting impact estimates of the relationship between identified candidate metabolites and OC phenotypes.

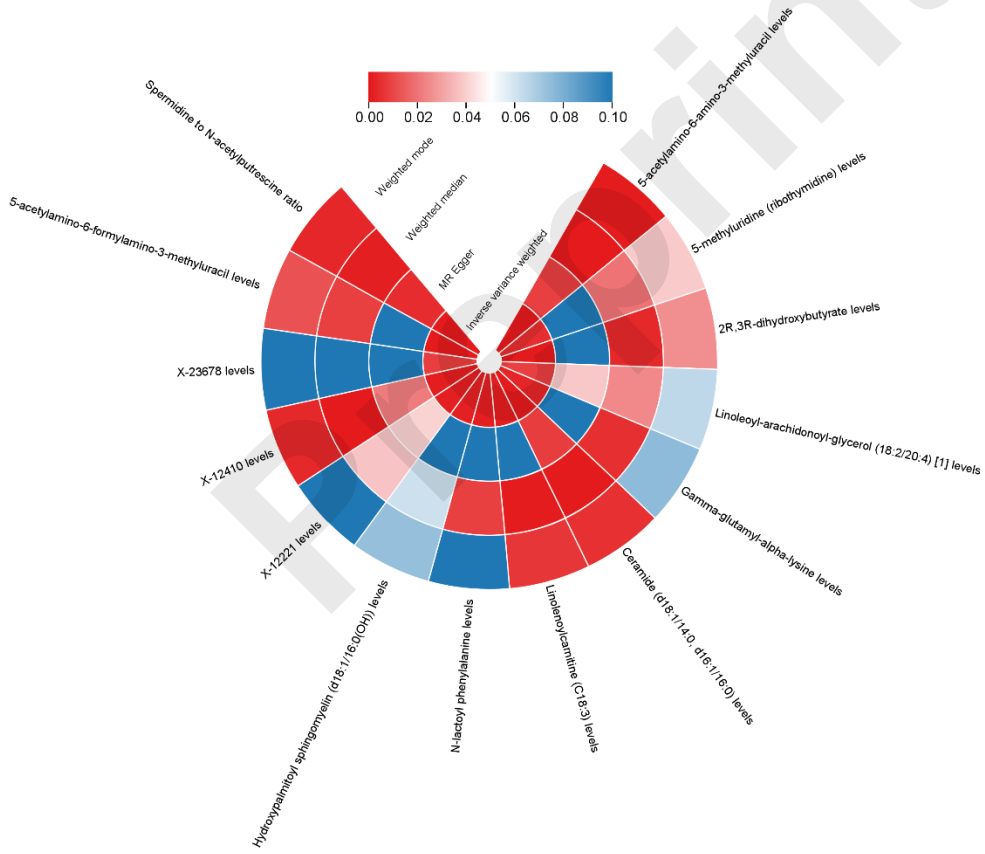

Figure 3 p value results of the four MR analyses for all positive results

Common metabolite phenotypes between OC and the five subtypes

Furthermore, we conducted the MR analysis involving a comprehensive assessment of 1,400 blood metabolites alongside five distinct subtypes of OC. This analysis was executed employing a consistent methodological framework, ensuring that

our approach-maintained uniformity across all the components of the study. Following the analysis of OC subtypes, a common pathogenic phenotype was identified for these subtypes, as illustrated in **Fig.4**.

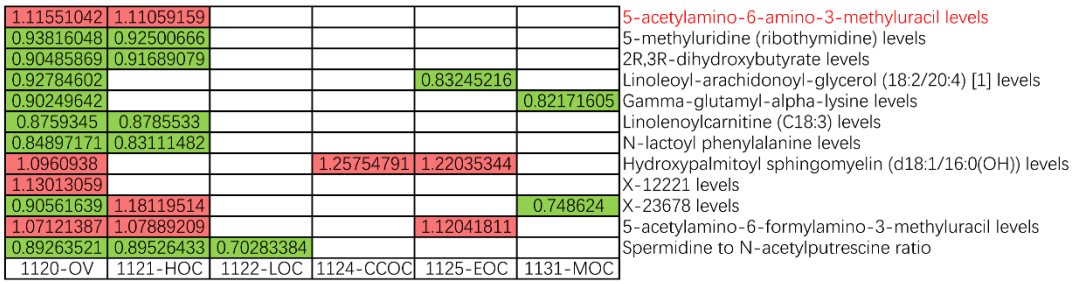

Figure 4 Shared metabolite phenotypes and OR values between OC and the five subtypes

Specific metabolite phenotypes in five OC subtypes

OC is classified into five main types: HOC, LOC, EOC, CCOC, MOC. HOC typically shows highly heterogeneous glandular structures, with CA125 and HE4 being common diagnostic markers, and p53 mutations are prevalent. EOC resembles endometrial glands, with positive ER and PR expression, and PTEN mutations are frequent. CCOC exhibits transparent vacuolated cells, with HNF1β as a specific marker and significant ARID1A mutations. MOC is characterized by mucin secretion, with elevated CEA levels, and KRAS mutations are common. Low-grade serous carcinoma has well-differentiated cells, frequent KRAS and BRAF mutations, and CA125 has limited diagnostic value.

High-grade serous ovarian cancer

With P<0.01, we screened 15 associated metabolites, 8 of which were identical to the OC results described above, and 7 were specific to HOC: trartronate (hydroxymalonate) level, N6-acetyllysine level, dopamine 3-o-sulfate level, 18:0/18:2-GPC level, PIP-Sulfate (2) level, 4-acetylcatechol sulfate (1) level, and adenosine 5'-monophosphate (AMP)-to-tyrosine ratio. These metabolites, when analyzed via four methods, produced results comparable to those obtained via IVW.

Low-grade serous ovarian cancer

Similar to HOC, we identified 9 known associated metabolites, with one overlapping with OC and 8 specific to LOC. These included 5 high-risk and 7 low-risk metabolites, which are as follows: sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1), sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0), the 3-formylindole level, the 3-indoleglyoxylic acid level, the alpha-ketoglutarate to aspartate ratio, the 2-o-methylascorbic acid level, the sucrose level, Spermidine to N-acetylputrescine ratio, and the adenosine 5'-monophosphate (AMP) to leucine ratio.

Endometeioid ovarian cancer

In addition, we screened 18 relevant known metabolites associated with EOC subtypes, of which 3 metabolites were identical to those associated with OC, and the remaining 15 metabolites were specific, including 9 high-risk metabolites: trimethylamine n-oxide level, 1,2-dilinoleoyl-GPC (18:2/18:2) level, 18:2/18:3-GPC level, tetradecadienedioate (C14:2-DC) level, SM(d18:1/16:0(OH)) levels, dimethylglycine level, AFAMU levels, retinol (vitamin A) to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio, cholesterol to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio, and 9 low-risk metabolites: glycocholenate sulfate level, 1-(1-enyl-stearoyl)-2-oleoyl-GPE (p-18:0/18:1) level, LAG (18:2/20:4) levels, dichosatrienoate (22:3n6) level, arachidonoylcarnitine (C20:4) levels, 2-ketocaprylate level, arachidonate (20:4n6) level, 18:0/20:4-GPC level, Spermidine to pyruvate ratio.

Clear cell ovarian cancer

There is one common outcome of OC, and there are also 3 specific high-risk metabolite, namely, 3-(3-hydroxyphenyl)propionate levels, SM(d18:1/16:0(OH)) levels, succinate levels, the cholesterol to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio, and 8 low-risk metabolites, namely, the quinate level, the taurocholenate sulfate level, the 4-hydroxycoumarin level, the 1-(1-enyl-palmitoyl)-GPC (p-16:0) ratio, the N-acetylcarnosine level, the 1-oleoyl-GPG (18:1) ratio, the arginine level, and the choline-to-choline ratio.

Mucinous ovarian cancer

Similarly, according to the IVW method, 15 known metabolites are thought to be associated with MOC, 2 of which are common to OC. Another 6 high-risk metabolites are specific to this phenotype: N-formylphenylalanine levels, N-methylhydroxyproline levels, 3-amino-2-piperidone levels, the adenosine 5'-diphosphate (ADP)-to-valine ratio, the isoleucine-to-phosphate ratio, and the leucine-to-phosphate ratio. In addition, 9 low-risk metabolites were identified: 4-hydroxyhippurate levels, Arabonate/xylonate levels, Enyl-16:0/18:1-GPE levels, GGAL levels, PIP-Sulfate(3) levels, Beta-hydroxyisovalerate levels, Guanidinoacetate levels, Orotidine levels, and the creatine to carnitine ratio.

Information on the results of MR analysis of OC subtypes can be found in the **Supplementary Table S4-S18 and Fig.S1-S5**.

Pathway enrichment analysis

As shown in Fig.4 (**Supplementary Table S19**), in our research, we conducted a thorough analysis of the metabolic pathways and enrichment of metabolites that are linked to OC and its various subtypes. This involved an investigation of the RaMP database, which yielded insightful results. Our findings revealed a total of 23 distinct metabolic pathways associated with OC. Notably, caffeine metabolism was the most significant among these pathways, highlighting its potential relevance in understanding the metabolic alterations associated with OC.

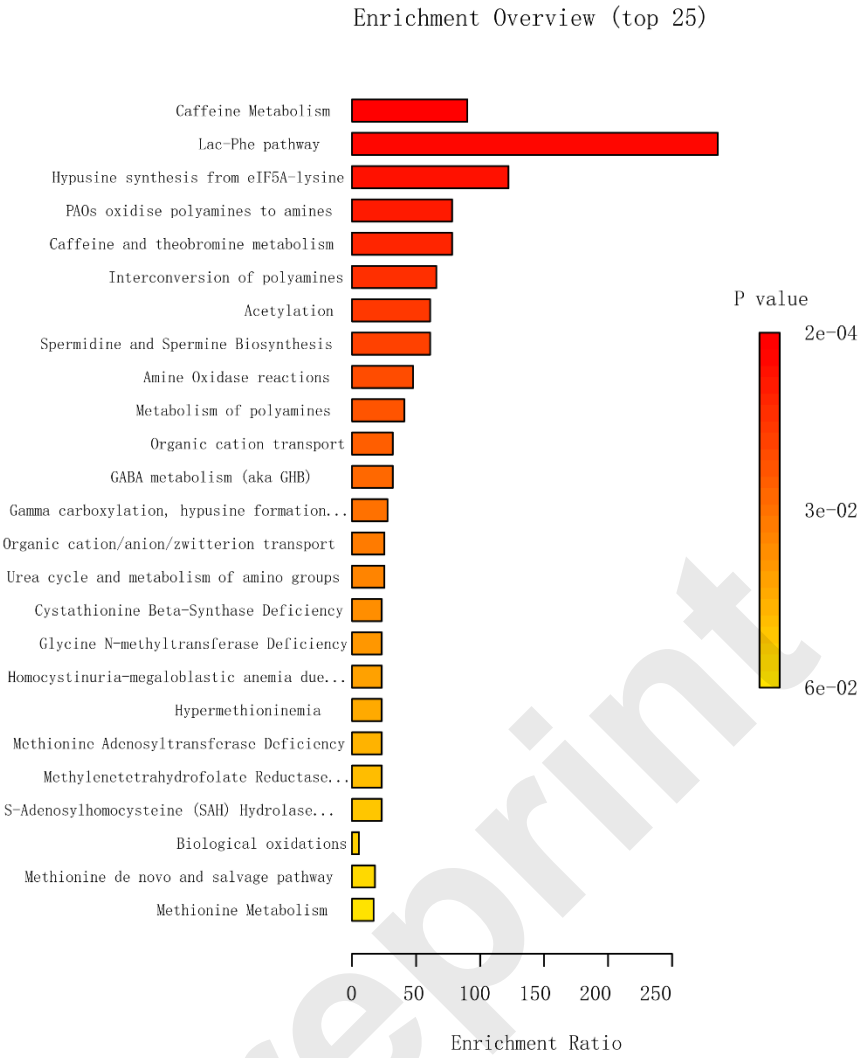

Figure 4 OC Metabolite Pathway Enrichment Top 25 Pathways.

The analysis of metabolic pathways across the five subtypes of OC revealed notable findings. Caffeine metabolism was significantly correlated with HOC. Moreover, pathways related to "Citrullinemia Type I," "Argininemia", "Argininosuccinic Aciduria", "Ornithine Transcarbamylase (OTC) Deficiency" and "Carbamoyl Phosphate Synthetase Deficiency" were strongly associated with LOC. The "methionine de novo and salvage" pathway was also significantly linked to EOC, whereas the "immune system" pathway was notably associated with CCOC. Furthermore, pathways involving mRNA, protein, and metabolite induction by cyclosporin A were significantly related to MOC. The most important pathways for each cancer subtype are presented in the **Supplementary Table S21-S24, Fig S1-S5**.

DISCUSSION:

In this study, eight high-risk factors and six low-risk factors were identified through genomics and metabolomics via MR. In addition, we reverse-validated our results,

which revealed no causality, eliminating bias associated with reverse causality and reinforcing the dependability of our preliminary MR result. After FDR correction, one high-risk factor for OC, AAMU, was identified. Moreover, metabolic pathway enrichment analysis revealed caffeine metabolism as the most significant pathway.

We characterized eight metabolites as potential cause-and-effect links to increased OC risk. Currently, research on the association between these metabolites and OC remains limited. LAG (18:2/20:4) and C18:3-Car participate in glycerophospholipid and lipid metabolism pathway. Lipid metabolism reprogramming is a signature of cancer, and studies have shown that certain polyunsaturated fatty acids (PUFAs) and their glycerides are linked to inflammation and cancer progression(39, 40). Carnitine derivatives, including linolenoylcarnitine, may be involved in mitochondrial energy metabolism and the metabolic adaptation of cancer cells(41). Our study further supports this perspective. GGAL, N-lactoylphenylalanine and 2R,3R-DHB are both involved in amino acid metabolism pathway. In cancer cells, amino acid metabolism is frequently reprogrammed to satisfy the elevated demands for nutrients, energy, and biosynthetic precursors necessary for growth. Studies have shown that N-lactoyl compounds may be linked to lactate metabolism and the acidification of the tumor microenvironment, allowing cancer cells to promote growth and immune evasion via this metabolic pathway(42-44). In future studies, we could explore targeting this pathway to disrupt immune evasion in tumor cells and thereby control cancer progression.

The ratio of spermidine to N-acetylputrescine can serve as an indicator of the balance between polyamine synthesis and degradation, which has been shown to be dysregulated in various types of cancer. Polyamine metabolism is often upregulated in cancer cells to support their rapid growth and survival(45, 46), making it a potential target for cancer therapy.

AAMU, m5U, and AFAMU are all involved in pyrimidine metabolism. Due to their essential role in cell proliferation, dysregulation of pyrimidine metabolism has already been recognized as a critical driver of tumorigenesis(47, 48).Cancer cells typically enhance pyrimidine synthesis (de novo synthesis) to meet the demands of rapid proliferation(49). Among these, m5U, a metabolic product of tRNA degradation, is linked to RNA methylation and is elevated in bladder cancer(50), potentially reflecting the high RNA metabolic activity of tumor cells. This finding aligns with our study. From a treatment perspective, targeting pyrimidine metabolism could provide a novel therapeutic approach which is that therapeutic strategies aimed at modulate pyrimidine metabolism could inhibit this excessive metabolic activity, potentially slowing tumor progression.

Our analysis revealed potential causal relationships between six metabolic products and the inhibition of OC progression. Among these, AAMU and AFAMU are involved in caffeine metabolism. Recent studies have shown that caffeine metabolism is closely linked to colorectal cancer(51), breast cancer(52), and prostate cancer(53), among others. The relationship between caffeine metabolism and OC risk may stem from the impact of caffeine and its metabolic byproducts on sex hormone regulation(54, 55). Studies have also demonstrated that caffeine can affect DNA methylation levels by inhibiting DNA methyltransferases (DNMTs), thus influencing the activity of

oncogenes and tumor suppressor genes(56). These findings align with our results, but the underlying mechanisms warrant further investigation. Future research should explore individual variations in caffeine metabolism to develop more targeted strategies for cancer prevention and treatment.

Ceramide (d18:1/14:0, d16:1/16:0) and SM(d18:1/16:0(OH)) are metabolites of sphingolipid metabolism. Both ceramide and sphingomyelin are critical tumor suppressors that regulate apoptosis, autophagy, and cell proliferation(57). Therefore, investigating the potential of sphingolipid metabolism, particularly the use of ceramide derivatives as anticancer agents, may provide a novel approach to cancer therapy.

Although five OC subtypes have unique metabolites distinct from those in other forms of OC, these notable findings do not lessen the importance of metabolites in the development of OC. There is growing evidence on observational studies has highlighted metabolic abnormalities in cancer patients relative to healthy individuals. These findings may guide the development of targeted treatment strategies for OC patients. These initial findings offer a foundation for further research.

We support the development of screening programs for groups displaying metabolic irregularities, employing big data analysis to highlight the significant impact of plasma metabolites on the clinical prevention and prognosis of OC. Currently, several methods, such as Mass Spectrometry, Nuclear Magnetic Resonance, are available for detecting plasma metabolite levels. Future studies could utilize ex vivo and in vivo experiments to further investigate the relationship between these metabolites and the diagnosis and treatment of OC. We hope to combine metabolic profiling with current diagnostic biomarkers (e.g., CA-125) in the future to improve diagnostic accuracy in OC. Furthermore, we endorse the practice of conducting longitudinal follow-up assessments of patients diagnosed with OC. This ongoing monitoring is crucial for exploring potential biomarkers associated with cancer recurrence. By tracking these patients over time, we can better understand the complex dynamics of cancer behavior and develop more effective strategies to predict and prevent recurrences.

Limitations:

Acknowledging the limitations of this study is essential. Firstly, the accurate MR analysis is contingent upon the explanation of IV exposure, emphasizes that larger sample sizes and more precise metabolomic measurements. Moreover, the study's focus on a European population restricts the generalizability of the findings, underscoring the importance of further validation in more diverse cohorts. Future studies should aim for more precise classification and characterization of phenotypes, as well as refinement of functional modeling to reduce bias. These improvements would enhance the power and validity of the results. Also, because MR can only be used to assess the causal effect of exposure on outcomes, without considering the quantification of potential causal effect sizes. Future studies should analyze longitudinal data and conduct in Clinical trials to validate the specific causal relationship between the expression levels of these

metabolites and OC, which will help determine whether these effects are age- and sex hormone-dependent or conditional on specific factors, thus providing a more nuanced understanding of the dynamic regulatory relationship between these metabolites and OC.

Conclusion:

This MR research highlights potential causal links between metabolites and OC, along with its subtypes. The results increase our understanding of OC development, including its different variants, and could serve as a foundation for improved management approaches in clinical practice. Nonetheless, because of insufficient strong supporting evidence, additional research is necessary to validate these connections and broaden these findings to increase their applicability in the early detection and diagnosis of OC. Future studies could investigate the combination of metabolites with existing diagnostic markers such as CA125 and HE4 to enhance the diagnostic accuracy for early-stage OC. Additionally, exploring novel therapeutic strategies targeting metabolites or metabolic pathways could offer promising approaches for inhibiting OC progression.

Abbreviations

OV: Ovarian cancer;
HOC: High grade serous ovarian cancer;
LOC: Low grade serous ovarian cancer;
CCOC: Clear cell ovarian cancer;
EOC: Endometrioid ovarian cancer;
MOC: Mucinous ovarian cancer;
ASR: Age-standardized rates;
MR: Mendelian randomization;
GWAS: Genomewide Association Study;
GDM: Genetically determined metabolites;
IVW: Inverse variance weighted;
FDR: False Discovery Rate;
SNP: Single Nucleotide Polymorphism;
KEGG: Kyoto Encyclopedia of Genes and Genomes;
RaMP: Relational Database of Metabolomic Pathways;
AAMU: 5-acetylamino-6-amino-3-methyluracil;
m5U: 5-methyluridine (ribothymidine);
2R,3R-DHB: 2R,3R-dihydroxybutyrate;
LAG (18:2/20:4): Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [1];
Enyl-16:0/18: 1-GPE:1-(1-enyl-palmitoyl)-2-oleoyl-GPE (p-16:0/18:1)
PIP-Sulfate(2): Sulfate of piperine metabolite C16H19NO3 (2)
PIP-Sulfate(3): Sulfate of piperine metabolite C16H19NO3 (3)
GGAL: Gamma-glutamyl-alpha-lysine;
C18:3-Car: Linolenoylcarnitine (C18:3);

SM(d18:1/16:0(OH)): Hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH));
AFAMU: 5-acetylamino-6-formylamino-3-methyluracil.
18:0/18:2-GPC:1-stearoyl-2-linoleoyl-gpc (18:0/18:2)
18:0/20:4-GPC:1-stearoyl-2-arachidonoyl-gpc (18:0/20:4)
18:2/18:3-GPC:1-linoleoyl-2-linolenoyl-GPC (18:2/18:3)

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Authors' contributions

The idea was conceived and proposed by JHW and HRF. Experiments performed and mansc written by HRF. YYS performed the visualization of the results. KY, LFZ and JXZ participated in data collection and interpretation. JHW reviewed and commented on the manuscript.

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Data availability

The plasma metabolites of MR were obtained from a study by Chen， the list of the summary indicators of GWASs focusing on plasma metabolites can obtained from GWAS Catalog (https://www.ebi.ac.uk/gwas/).
MR data of ovarian cancer and each subtype of ovarian cancer were obtained from IRU open GWAS (https://gwas.mrcieu.ac.uk/).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict interests

The authors declare no conflicts of interest.

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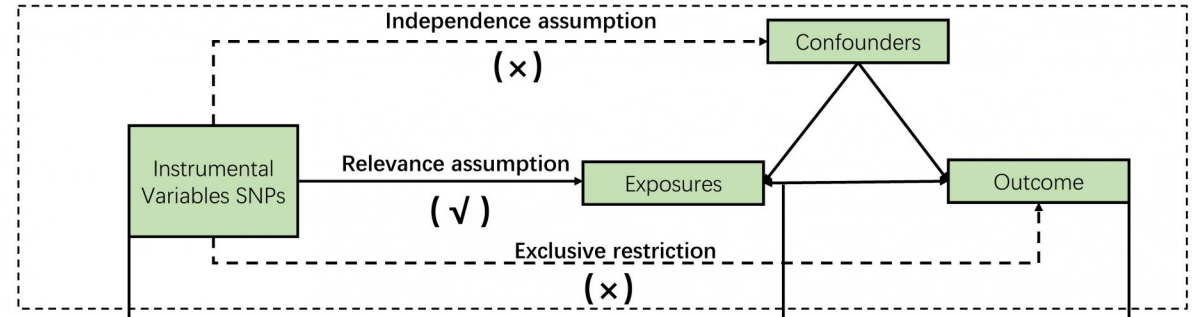
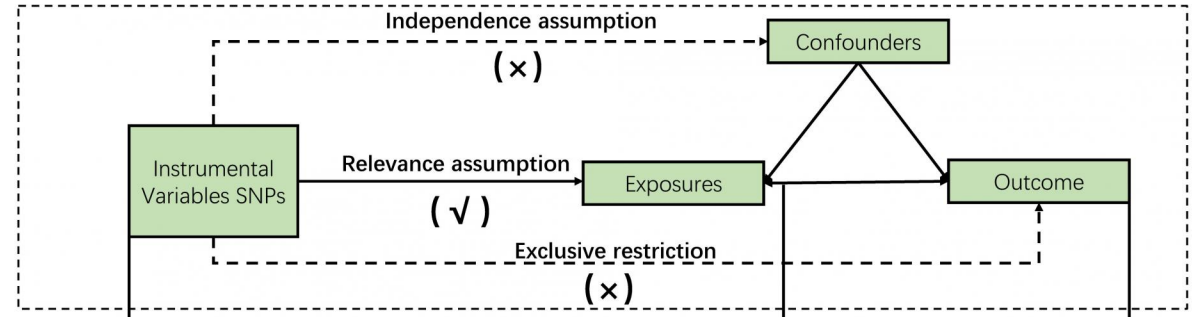


Selection criteria for 1400 serum Metabolites:
1.SNP associated with exposure at $p<1\times10^{-5}$;
2.LD $r^2<0.01$ within 500 kb distance;
3.F statistic > 10 is considered for selection of strong IVs.

Primary analysis: IVW
Complementary analyses: MR-Egger, weighted median, and weighted mode
Sensitive analyses: Cochran Q test, MR-Egger intercept, MR-PRESSO

Extract detailed information SNPs for ovarian cancer and its subtypes:
Ieu-a-1120 OC
ieu-a-1121 HOC
Ieu-a-1122 LOC
ieu-a-1224 CCOC
ieu-a-1125 EOC
ieu-a-1231 MOC

GWAS ID	Year	Trait	sample size	Ncase
ieu-a-1120	2017	OV	66450	25509
ieu-a-1121	2017	HOC	53978	13037
ieu-a-1122	2017	LOC	41953	1012
ieu-a-1124	2017	CCOC	42307	1366
ieu-a-1125	2017	EOC	43751	2801
ieu-a-1231	2017	MOC	43507	2566



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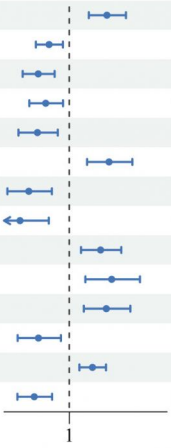
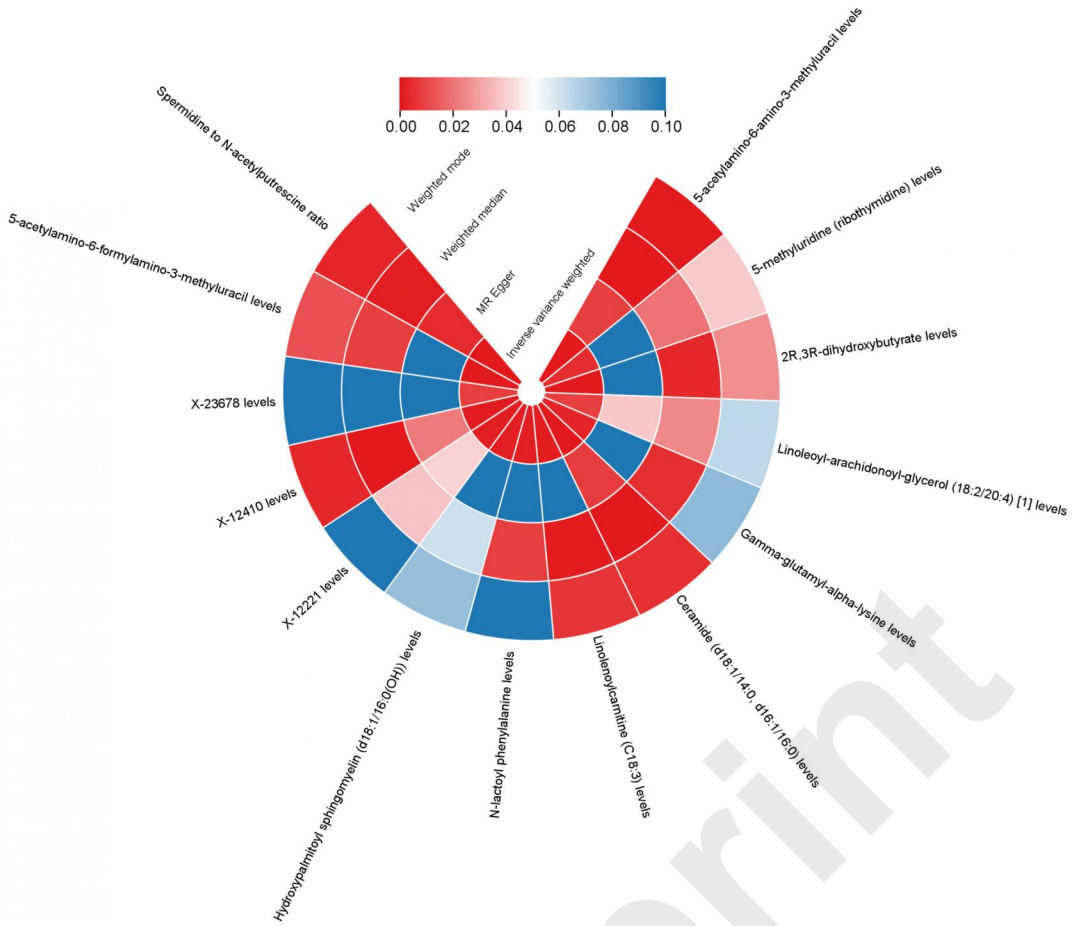
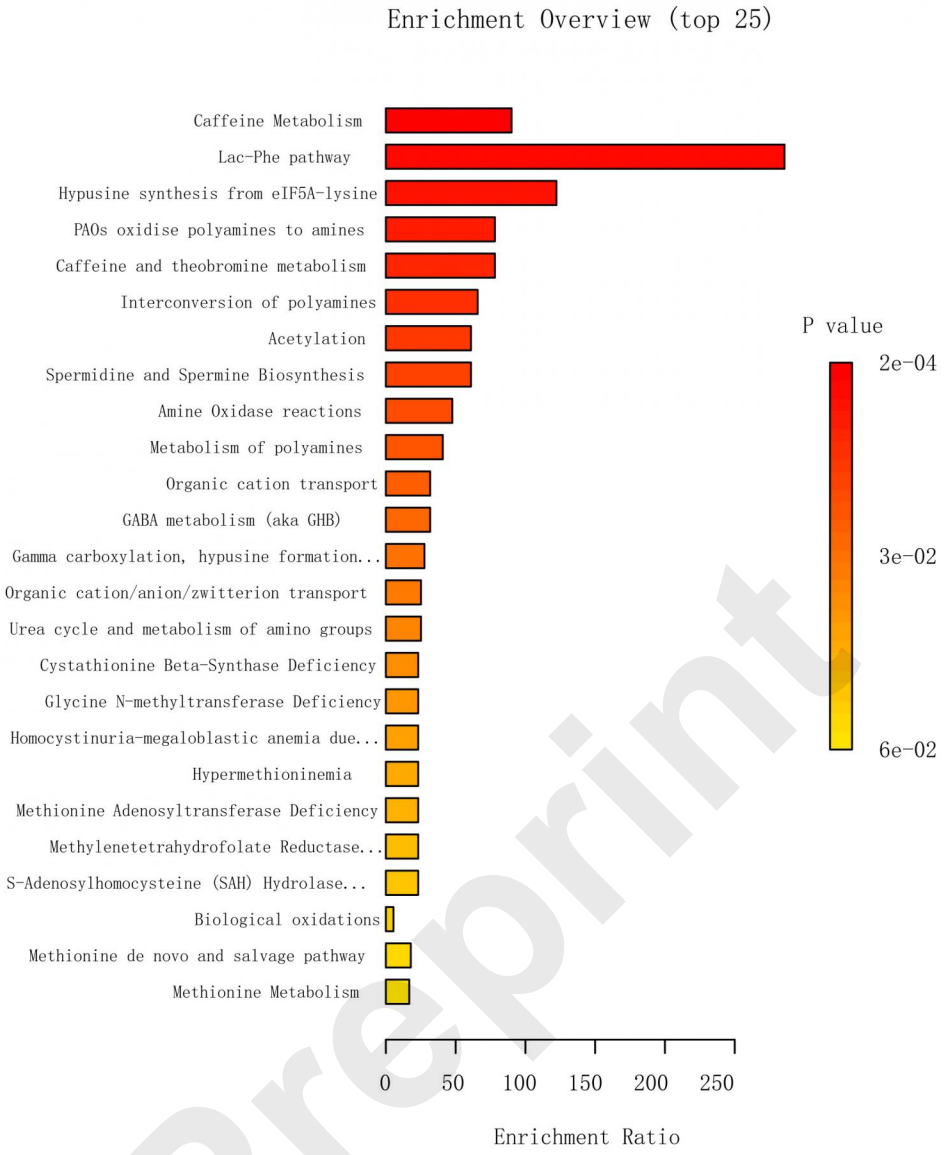
Ieu-a-1122 LOC
ieu-a-1224 CCOC
ieu-a-1125 EOC
ieu-a-1231 MOC

Exposure	No.of SNP	Method	OR(95% CI)	P
5−acetylamino−6−amino−3−methyluracil levels	34	Inverse variance weighted	1.12 (1.06 to 1.17)	0.000
5−methyluridine (ribothymidine) levels	25	Inverse variance weighted	0.94 (0.90 to 0.98)	0.005
2R,3R−dihydroxybutyrate levels	30	Inverse variance weighted	0.90 (0.86 to 0.96)	0.000
Linoleoyl−arachidonoyl−glycerol (18:2/20:4) [1] levels	19	Inverse variance weighted	0.93 (0.88 to 0.98)	0.008
Gamma−glutamyl−alpha−lysine levels	31	Inverse variance weighted	0.90 (0.84 to 0.96)	0.003
Ceramide (d18:1/14:0, d16:1/16:0) levels	26	Inverse variance weighted	1.12 (1.05 to 1.19)	0.000
Linolenoylcarnitine (C18:3) levels	21	Inverse variance weighted	0.88 (0.81 to 0.95)	0.001
N−lactoyl phenylalanine levels	16	Inverse variance weighted	0.85 (0.77 to 0.94)	0.001
Hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH)) levels	31	Inverse variance weighted	1.10 (1.04 to 1.16)	0.001
X−12221 levels	23	Inverse variance weighted	1.13 (1.05 to 1.22)	0.001
X−12410 levels	25	Inverse variance weighted	1.11 (1.04 to 1.19)	0.001
X−23678 levels	19	Inverse variance weighted	0.91 (0.84 to 0.97)	0.008
5−acetylamino−6−formylamino−3−methyluracil levels	22	Inverse variance weighted	1.07 (1.03 to 1.11)	0.000
Spermidine to N−acetylputrescine ratio	20	Inverse variance weighted	0.89 (0.84 to 0.95)	0.000




0.00
0.02
0.04
0.06
0.08
0.10
Weighted mode
Weighted median
MR Egger
Inverse variance weighted
Spermidine to N-acetylputrescine ratio
5-acetylamino-6-formylamino-3-methyluracil levels
X-23678 levels
X-12410 levels
X-12221 levels
Hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH)) levels
N-lactoyl phenylalanine levels
Linolenoylcarnitine (C18:3) levels
Ceramide (d18:1/14:0, d16:1/16:0) levels
Gamma-glutamyl-alpha-lysine levels
Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [1] levels
2R,3R-dihydroxybutyrate levels
5-methyluridine (ribothymidine) levels
5-acetylamino-6-amino-3-methyluracil levels

1.11551042	1.11059159					5-acetylamino-6-amino-3-methyluracil levels
0.93816048	0.92500666					5-methyluridine (ribothymidine) levels
0.90485869	0.91689079					2R,3R-dihydroxybutyrate levels
0.92784602				0.83245216		Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [1] levels
0.90249642					0.82171605	Gamma-glutamyl-alpha-lysine levels
0.8759345	0.8785533					Linolenoylcarnitine (C18:3) levels
0.84897171	0.83111482					N-lactoyl phenylalanine levels
1.0960938			1.25754791	1.22035344		Hydroxypalmitoyl sphingomyelin (d18:1/16:0(OH)) levels
1.13013059						X-12221 levels
0.90561639	1.18119514				0.748624	X-23678 levels
1.07121387	1.07889209			1.12041811		5-acetylamino-6-formylamino-3-methyluracil levels
0.89263521	0.89526433	0.70283384				Spermidine to N-acetylputrescine ratio
1120-OV	1121-HOC	1122-LOC	1124-CCOC	1125-EOC	1131-MOC	


Enrichment Overview (top 25)
Caffeine Metabolism
Lac-Phe pathway
Hypusine synthesis from eIF5A-lysine
PAOs oxidise polyamines to amines
Caffeine and theobromine metabolism
Interconversion of polyamines
Acetylation
Spermidine and Spermine Biosynthesis
Amine Oxidase reactions
Metabolism of polyamines
Organic cation transport
GABA metabolism (aka GHB)
Gamma carboxylation, hypusine formation...
Organic cation/anion/zwitterion transport
Urea cycle and metabolism of amino groups
Cystathionine Beta-Synthase Deficiency
Glycine N-methyltransferase Deficiency
Homocystinuria-megaloblastic anemia due...
Hypermethioninemia
Methionine Adenosyltransferase Deficiency
Methylenetetrahydrofolate Reductase...
S-Adenosylhomocysteine (SAH) Hydrolase...
Biological oxidations
Methionine de novo and salvage pathway
Methionine Metabolism
0
50
100
150
200
250
Enrichment Ratio
P value
2e-04
3e-02
6e-02