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ORIGINAL RESEARCH

Metabolomics Reveals Glyoxylate and Dicarboxylate Metabolism Disorder in Elderly Trauma: A Retrospective Study

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Background: Elderly trauma (ET) carries a high mortality rate due to comorbidities, frailty, and limited physiological reserve. Understanding its specific pathophysiology is essential for enabling precision treatment.

Objective: To identify characteristic metabolic dysregulations and specific pathways in geriatric trauma.

Methods: We retrospectively analyzed existing metabolomics data from ET, young and middle-aged trauma (YMAT), elderly controls (EC), and young and middle-aged controls (YMAC).

Results: An IVD integrating 8 significant metabolic pathways (SMPs) from ET vs EC and 10 SMPs from YMAT vs YMAC identified 3 pathways specific to ET: glyoxylate and dicarboxylate metabolism, galactose metabolism, and Pantothenate and coenzyme A (CoA) biosynthesis. The second IVD integrating these pathways with 3 SMPs from EC vs YMAC identified 2 metabolic pathways specific to ET independent of natural aging: glyoxylate and dicarboxylate metabolism, and pantothenate and CoA biosynthesis. Finally, the third IVD integrating them and 7 SMPs from ET vs YMAT identified glyoxylate and dicarboxylate metabolism as unique signature of geriatric trauma.

Conclusion: This study was one of few metabolomics studies that distinguish between geriatric trauma-related metabolic changes and baseline aging factors, and revealed glyoxylate and dicarboxylate metabolism disorder as a key pathway specific to geriatric trauma. Understanding it may inform the development of age-tailored strategies for improving trauma outcomes in the elderly.

Keywords: elderly trauma, metabolomics, metabolic pathways, glyoxylate and dicarboxylate metabolism

Introduction

The global population is aging rapidly. Worldwide, the number of people aged 60 or older is projected to reach 2 billion by 2050.[1] The proportion of adults aged 65 and older is expected to grow from 16.8% in 2020 to 20% by 2040.[2] Similarly, China's population aged 60 and above reached 267.36 million (18.9% of the total) by the end of 2021 and is projected to soar to 400 million (30%) by 2035.[3,4] Concurrent with this demographic shift, geriatric trauma now constitutes 30–50% of all trauma cases and its incidence continues to rise.[5–7] Due to comorbidities, frailty, and diminished physiological reserve, older trauma patients typically experience prolonged hospitalizations and rehabilitation, substantially higher healthcare costs, and significantly elevated mortality rates, which poses immense challenges to families, healthcare systems, and social security networks.[8–10] Comorbidities are highly prevalent in geriatric trauma, affecting approximately 80% of patients, with common conditions including hypertension, arthritis, heart disease, diabetes, and a history of stroke.[10] They synergistically increase vulnerability to physiological stress and worsen post-traumatic complications. Clinically, the unique challenges of this population are gaining recognition globally. For

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instance, the 2023 WSES guidelines by De Simone et al[9] provide evidence-based protocols to optimize care and reduce unnecessary interventions. Furthermore, Pawan Acharya et al[11] have refined the Geriatric Trauma Outcome Score to improve mortality prediction. Despite these clinical advances, fundamental research into the underlying pathophysiological mechanisms of geriatric trauma remains critically scarce.

Metabolomics provides a comprehensive quantitative profile of all detectable metabolites (molecular weight < 1500 Da) in a biological sample, encompassing diverse classes such as peptides, lipids, amino acids, and carbohydrates.[12] As the ultimate substrates and products of metabolic processes, these small molecules offer a dynamic snapshot of endogenous physiological states—influenced by genetics and transcription—and exogenous exposures.[13] They are integral to pivotal cellular functions, including energy production, signal transduction, and apoptosis. Consequently, metabolomics has become an indispensable tool for discovering biomarkers, elucidating systemic metabolic alterations, mapping underlying regulatory pathways, and identifying bioactive metabolites that directly influence phenotypic outcomes.[13–15] Our prior metabolomic research has successfully identified five early predictive biomarkers for post-traumatic sepsis (each with an AUC > 0.94) and implicated glycerophospholipid, porphyrin, and sphingolipid metabolism in its pathogenesis.[14] Furthermore, we discovered five additional biomarkers capable of distinguish post-traumatic sepsis from trauma-induced systemic inflammatory response syndrome (AUC > 0.94 for each).[15] Youjin Chang et al[12] used metabolomics to reveal distinct metabolic patterns between direct and indirect subtypes of sepsis-induced acute respiratory distress syndrome. Building upon this foundation, applying metabolomics to characterize the metabolic profile of ET holds substantial promise for uncovering its unique biological mechanisms. More importantly, elucidating these distinct characteristics will be invaluable for advancing personalized, precision treatments and ultimately improving clinical outcomes in this population. Despite extensive literature on clinical outcomes and prognostic scores in elderly trauma, significant gaps remain in our understanding of its fundamental biological mechanisms.[16,17] Our study is designed to elucidate the distinct metabolic signatures of geriatric trauma, thereby providing a scientific basis for development of age-tailored strategies for improving trauma outcomes in the elderly.

Method

Study Design and Patient Selection

This study adhered to the ethical standards outlined in the Helsinki Declaration of 1964 and its later amendments and was approved by the Medical Ethics Committee of General Hospital of Ningxia Medical University (KYLL-2025-1113). The requirement for informed consent was waived by the committee due to the retrospective nature of the study and the use of existing data. The inclusion criteria were: 1) Injury severity score (ISS) ≥16, 2) age≥18 years, 3) admission to the emergency department within 24 hours post-trauma. Exclusion criteria were: 1) Pregnant or lactating women, 2) pre-existing immunodeficiency, ongoing immunosuppressive therapy, or malignancy. The final cohort comprised existing metabolomics data from 20 elderly trauma (ET, age ≥ 60 years) patients, 34 young and middle-aged trauma (YMAT, 18 ≤ age ≤ 55 years) patients, 7 elderly controls (EC), and 33 young and middle-aged controls (YMAC). Plasma samples were obtained from trauma patients admitted to the General Hospital of Ningxia Medical University within 24 hours post-injury. Blood collection was performed within one hour of admission between March 2022 and November 2023. Venous blood was drawn into disposable EDTA-K2 vacuum tubes and processed within 15 minutes. The samples were centrifuged at 3000 rpm for 10 minutes at room temperature. The resulting plasma supernatant was aliquoted and stored at −80°C for approximately eight months prior to metabolomic analysis. First, metabolic pathways specific to geriatric trauma were identified by comparing ET and EC groups and YMAT and YMAC groups. Subsequently, a comparison of EC vs YMAC was conducted to exclude the effects of aging per se, thereby isolating geriatric trauma-specific pathways. Finally, validation via direct comparison of ET vs YMAT confirmed the geriatric trauma-specific metabolic signatures identified in the prior steps.

Metabolomics Workflow

Sample Preparation and Metabolite Extraction

The samples (100 μL) were placed in the EP tubes and resuspended with prechilled 80% methanol by well vortex. Then the samples were incubated on ice for 5 min and centrifuged at 15,000 g, 4°C for 20 min. Some of supernatant was

diluted to final concentration containing 53% methanol by LC-MS grade water. The samples were subsequently transferred to a fresh Eppendorf tube and then were centrifuged at 15000 g, 4°C for 20 min. Finally, the supernatant was injected into the LC-MS/MS system analysis.

UHPLC-MS/MS Analysis

UHPLC-MS/MS analyses were performed using a Vanquish UHPLC system (ThermoFisher, Germany) coupled with an Orbitrap Q ExactiveTM HF mass spectrometer or Orbitrap Q ExactiveTMHF-X mass spectrometer (Thermo Fisher, Germany) in GenechemCo., Ltd. (Shanghai China). Samples were injected onto a Hypersil Goldcolumn (100×2.1 mm, 1.9μm) using a 12-min linear gradient at a flow rate of 0.2 mL/min. The eluents for the positive and negative polarity modes were eluent A (0.1% FA in Water) and eluent B (Methanol). The solvent gradient was set as follows: 2% B, 1.5 min; 2–85% B, 3 min; 85–100% B, 10 min; 100–2% B, 10.1 min; 2% B, 12 min. Q ExactiveTM HF mass spectrometer was operated in positive/negative polarity mode with spray voltage of 3.5 kV, capillary temperature of 320°C, sheath gas flow rate of 35 psi and aux gas flow rate of 10 L/min, S-lens RF level of 60, Aux gas heater temperature of 350°C.

Data Processing and Metabolite Identification

The raw data files generated by UHPLC-MS/MS were processed using the Compound Discoverer 3.3 (CD3.3, ThermoFisher) to perform peak alignment, peak picking, and quantitation for each metabolite. The main parameterswere set as follows: peak area was corrected with the first QC, actual mass tolerance, 5ppm; signal intensity tolerance, 30%; and minimum intensity, et al. After that, peak intensities were normalized to the total spectral intensity. The normalized data was used to predict the molecular formula based on additive ions, molecular ion peaks and fragment ions. And then peaks were matched with the mzCloud, mzVault and MassList database to obtain the accurate qualitative and relative quantitative results. Compounds whose CVs of relative peak areas in QC samples were greater than 30% were removed, and finally the metabolites' identification and relative quantification results were obtained. These metabolites were annotated using the KEGG database, HMDB database and LIPIDMaps database.

Data Collection

Comprehensive clinical data were obtained on the first day of emergency department admission, which included: Age, gender, ISS, GCS, white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin (HGB), platelet (PLT), albumin (Alb), fibrinogen (FIB), D-dimer, lactic acid (LAC), past medical history, injury mechanism, injury site.

Statistical Analysis

Statistical analyses were performed using IBM SPSS (version 26.0). Continuous variables were expressed as mean ± standard deviation for normally distributed data and as median (interquartile range, 25–75%) for non-normally distributed data. Independent sample t-tests were used for normally distributed data, while the Mann–Whitney test was used for non-normally distributed data. Categorical variables were presented as numbers and percentages, and Pearson's χ^2 test or Fisher's exact test was used for group comparisons. For all analyses, a two-tailed p-value<0.05 was considered statistically significant. Metabolomics data was normalized, log-transformed, and auto-scaled to generate more comparable individual features prior to the statistical analyses. Partial least-squares discriminant analysis (PLS-DA), a heatmap using Euclidean and *T*-test, and pathway analysis were performed using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/). The differentially expressed metabolites (DEMs) were identified based on the criteria of variable importance in projection (VIP) score >1.0 and false discovery rate (FDR) < 0.05. Interactive Venn diagram (IVD) was performed using EVenn (http://ehbio.com/test/venn/#/). Pathways with *P*-value<0.05 were considered significant.

Results

Patients' Characteristics

Table 1 showed no significant differences in age and gender between the ET and EC groups ($P > 0.05$). Compared with the EC group, the ET group exhibited significantly higher WBC and ANC, while HGB and Alb levels were significantly

Table 1 A Comparison of the Characteristics of the ET and EC Groups

Characteristics	ET (N=20)	EC (N=7)	χ^2/t/Z	*P*-value
Demographic data				
Age	69.3±5.8	69.6±7.3	−0.118	0.907
Male (n, %)	13 (65.0)	7 (100.0)		0.137
Assessment of injury severity				
ISS	19.0 (17.0–32.0)	NA		
GCS	13.0 (6.0–15.0)	NA		
Laboratory test results				
WBC (10^3/mm^3)	18.2±5.0	7.6±2.3	5.368	<0.001
ANC (10^3/mm^3)	15.1±4.3	4.5±1.7	6.240	<0.001
HGB (g/L)	126.5±25.1	155.4±6.3	−2.983	0.006
PLT (10^3/mm^3)	191.3±52.7	220.6±69.1	−1.167	0.254
Alb (g/L)	35.3±5.7	43.1±2.9	−3.448	0.002

Abbreviations: ET, elderly trauma; EC, elderly control; ISS, injury severity score; NA, not applicable, as EC group did not undergo assessment of injury severity; GCS, Glasgow Coma Scale; WBC, white blood cell; ANC, absolute neutrophil count; HGB, hemoglobin; PLT, platelet; Alb, albumin.

lower ($P < 0.05$). Similarly, Table 2 showed no significant differences in age and gender between the YMAT and YMAC groups ($P > 0.05$). Compared with the YMAC group, the YMAT group exhibited significantly higher WBC and ANC, while HGB and Alb levels were significantly lower ($P < 0.05$). Baseline characteristics in Table 3 revealed no significant differences in gender, ISS, GCS, injury mechanism, and injury sites between the ET and YMAT groups ($P > 0.05$), while the proportions of primary hypertension and type 2 diabetes were significantly different between the two groups. The comparison of baseline characteristics between EC and YMAC group were shown in Table 4.

ET Group vs EC Group

This primary comparison group forms the basis for identifying plasma metabolite alterations specific to geriatric trauma. By contrasting ET with EC, we isolate trauma-induced metabolic changes in the geriatric population, thereby facilitating the screening of DEMs directly associated with geriatric trauma. When analyzing the ET and EC groups using PLS-DA, 2D PLS-DA score plot based on the abundance profile of the 1573 metabolites demonstrated distinct separation between the ET and EC groups (Figure 1A), and we chose 5 component which were achieved by cross-validation method of PLS-DA with R^2 =0.99991, Q^2 =0.96091, and accuracy of 1.0 (Figure 1A). A high R^2 value close to 1 indicates an excellent fit

Table 2 A Comparison of the Baseline Data of the YMAT and YMAC Groups

Characteristics	YMAT (N=34)	YMAC (N=33)	χ^2/t	*P*-value
Demographic data				
Age	38.4±11.0	39.7±11.0	−0.467	0.642
Male (n, %)	29 (85.3)	23 (69.7)	2.345	0.126
Assessment of disease severity				
ISS	19.0 (17.0–32.0)	NA		
GCS	13.0 (6.0–15.0)	NA		
Laboratory test results				
WBC (10^3/mm^3)	19.2±6.8	7.1±1.8	9.825	<0.001
ANC (10^3/mm^3)	16.9±6.2	3.9±1.3	11.806	<0.001
HGB (g/L)	118.4±25.4	156.0±17.2	−7.081	<0.001
PLT (10^3/mm^3)	209.0±68.6	246.9±78.9	−2.101	0.040
Alb (g/L)	33.6±8.6	46.6±2.7	−8.289	<0.001

Abbreviations: YMAT, young and middle-aged trauma; YMAC, young and middle-aged control; ISS, injury severity score; NA, not applicable, as YMAC group did not undergo assessment of injury severity; GCS, Glasgow Coma Scale; WBC, white blood cell; ANC, absolute neutrophil count; HGB, hemoglobin; PLT, platelet; Alb, albumin.

Table 3 A Comparison of the Baseline Data of the ET and YMAT Groups

Characteristics	ET (N=20)	YMAT (N=34)	χ^2/t/Z	*P*-value
Demographic data				
Age	69.3±5.8	38.4±11.0	11.561	<0.001
Male (n, %)	13 (65.0)	29 (85.3)	1.941	0.164
Assessment of injury severity				
ISS	19.0 (17.0–32.0)	26.0 (17.0–34.0)	−1.070	0.285
GCS	13.0 (6.0–15.0)	10.0 (6.0–15.0)	−0.424	0.672
Laboratory test results				
WBC (10^3/mm^3)	18.2±5.0	19.2±6.8	−0.564	0.575
ANC (10^3/mm^3)	15.1±4.3	16.9±6.2	−1.135	0.261
HGB (g/L)	126.5±25.1	118.4±25.4	1.137	0.261
PLT (10^3/mm^3)	191.3±52.7	209.0±68.6	−0.994	0.325
Alb (g/L)	35.3±5.7	33.6±8.6	0.778	0.440
FIB (g/L)	1.8 (1.3–2.9)	2.2 (1.6–2.9)	−0.618	0.537
D-dimer (ug/mL)	55.5 (32.0–100.8)	26.0 (7.5–77.0)	−1.810	0.070
LAC (mmol/L)	2.9 (1.7–6.5)	3.5 (1.7–4.8)	−0.502	0.616
Comorbid diseases				
Primary hypertension (n, %)	8 (40.0)	2 (5.9)	7.585	0.006
Type 2 diabetes (n, %)	6 (30.0)	1 (2.9)	5.950	0.015
Injury mechanism (n, %)				0.474
Motor vehicle accident	17 (85.0)	24 (70.6)		
Fall	3 (15.0)	8 (23.5)		
Others	0 (0.0)	2 (5.9)		
Site of injury				
Head and neck (n, %)	18 (90.0)	24 (70.6)	1.737	0.188
Face (n, %)	5 (25.0)	12 (35.3)	0.619	0.432
Chest (n, %)	13 (65.0)	29 (85.3)	1.941	0.164
Abdomen (n, %)	9 (45.0)	16 (47.1)	0.021	0.884
Limbs and pelvis (n, %)	9 (45.0)	17 (50.0)	0.126	0.723

Abbreviations: ET, elderly trauma; YMAT, young and middle-aged trauma; ISS, injury severity score; GCS, Glasgow Coma Scale; WBC, white blood cell; ANC, absolute neutrophil count; HGB, hemoglobin; PLT, platelet; Alb, albumin; FIB, fibrinogen; LAC, lactic acid.

Table 4 A Comparison of the Baseline Data of the EC and YMAC Groups

Characteristics	EC (N=7)	YMAC (N=33)	χ^2/t/Z	*P*-value
Demographic data				
Age	69.6±7.3	39.7±11.0	6.851	<0.001
Male (n, %)	7 (100.0)	23 (69.7)	1.443	0.230
Laboratory test results				
WBC (10^3/mm^3)	7.6±2.3	7.1±1.8	0.636	0.529
ANC (10^3/mm^3)	4.5 (3.8–4.6)	3.5 (3.1–4.5)	−1.139	0.255
HGB (g/L)	155.4±6.3	156.0±17.2	−0.081	0.936
PLT (10^3/mm^3)	220.6±69.1	246.9±78.9	−0.818	0.418
Alb (g/L)	43.1±2.9	46.6±2.7	−3.034	0.004

Abbreviations: EC, elderly control; YMAC, young and middle-aged control; WBC, white blood cell; ANC, absolute neutrophil count; HGB, Hemoglobin; PLT, platelet; Alb, albumin.

of the model to the data, and a high Q^2 value suggests good predictive ability of the model. PLS-DA identified 558 metabolites with VIP score > 1, the top 15 metabolites based on VIP score were shown in Figure 1B. *T*-test identified 625 metabolites with FDR < 0.05 (Figure 1C), ultimately leading to the identification of 558 DEMs. By VIP and *t*-test, a heatmap was constructed to provide an intuitive visualization of the discriminant metabolites, which showed metabolite

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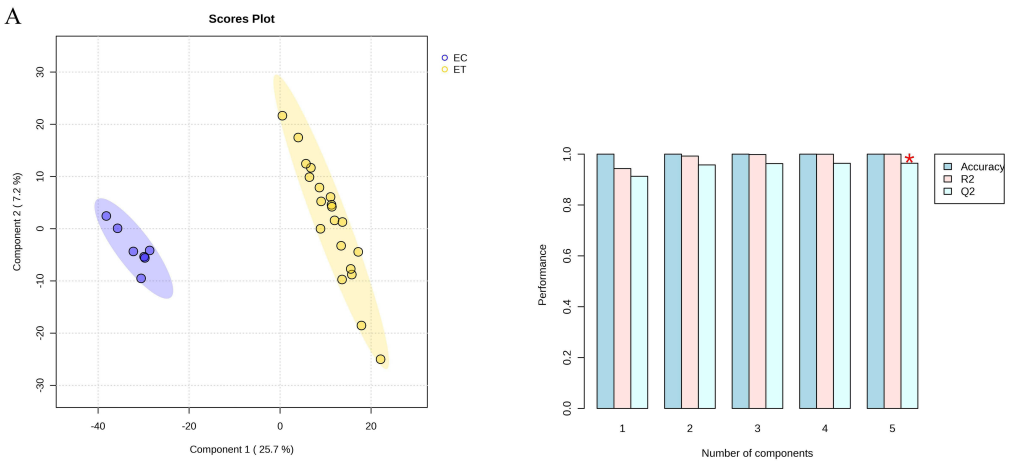
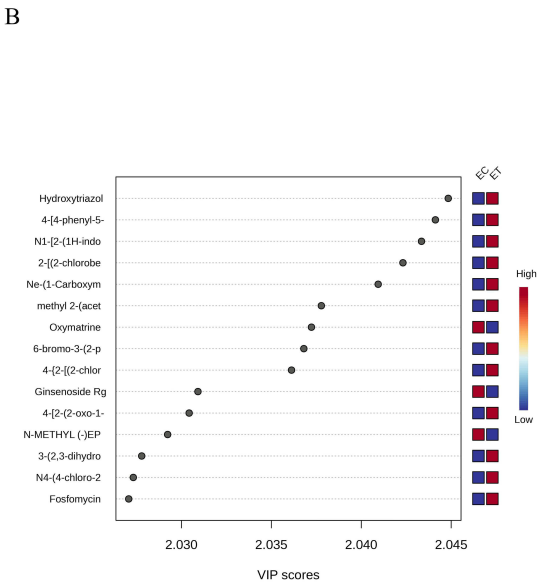
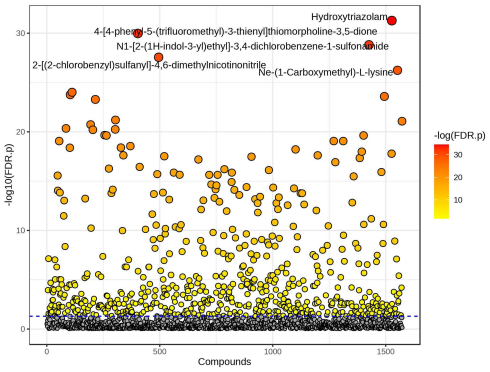


PLS-DA cross validation details:

Measure	1 comps	2 comps	3 comps	4 comps	5 comps
Accuracy	1.0	1.0	1.0	1.0	1.0
R^2	0.94328	0.99239	0.99829	0.99968	0.99991
Q^2	0.9137	0.95993	0.95998	0.96389	0.96091





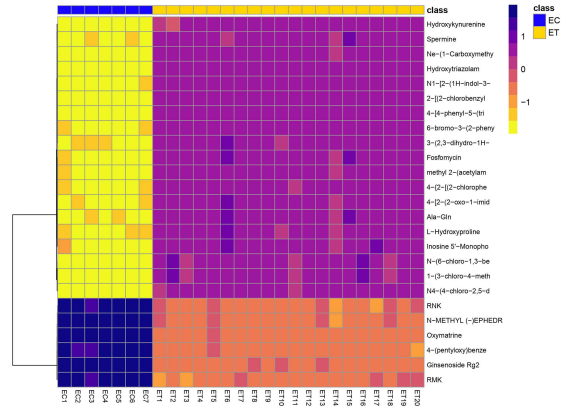


Figure 1 Screening for DEMs Between the ET and EC groups. (**A**) PLS-DA; (**B**) The top 15 metabolites discriminating the two groups (based on VIP score); (**C**) *t*-test; (**D**) Hierarchical heatmap for top-25 discriminating metabolites. ★ selected principal component based on the largest Q^2.

Abbreviations: ET, elderly trauma; EC, elderly control; PLS-DA, partial least-squares discriminant analysis; VIP, variable importance in projection; FDR, false discovery rate.

levels were significantly different between the ET and EC groups (top 25) (Figure 1D), indicating potential biomarkers for differentiating the two conditions. The top 15 DEMs (based on VIP) are shown in sTable 1.

YMAT Group vs YMAC Group

This contrast aims to identify alterations in the plasma metabolome of young adults following trauma. Given the distinct physiological functions between young and elderly populations, this group serves as a control reference for the geriatric trauma cohort, thereby facilitating an understanding of the influence of age-related factors on the traumatic response. 2D PLS-DA score plot based on the abundance profile of the 1573 metabolites demonstrated distinct separation between the YMAT and YMAC groups (Figure 2A), and cross-validation of PLS-DA revealed that the model exhibited excellent explanatory and predictive capabilities (Figure 2A). PLS-DA identified 554 metabolites with VIP score > 1, the top 15 metabolites based on VIP score were shown (Figure 2B). *t*-test identified 945 metabolites with FDR < 0.05 (Figure 2C), ultimately leading to the identification of 554 DEMs. By VIP and *t*-test, a heatmap showed metabolite levels were significantly different between the YMAT and YMAC groups (top 25) (Figure 2D). The top 15 DEMs (based on VIP) are shown in sTable 2.

EC Group vs YMAC Group

Comparing these two groups serves to identify baseline differences in the plasma metabolome between trauma-free elderly and young adult populations. This thereby provides contextual information to interpret intergroup differences between the geriatric trauma and young adult trauma cohorts, enabling differentiation of age-related baseline variations from trauma-specific alterations. 2D PLS-DA score plot based on the abundance profile of the 321 metabolites demonstrated distinct separation between the EC and YMAC groups (Figure 3A), and cross-validation of PLS-DA revealed that the model exhibited moderate predictive power but relatively high classification accuracy. (Figure 3A). PLS-DA identified 126 metabolites with VIP score > 1, the top 15 metabolites based on VIP score were shown (Figure 3B). *T*-test identified 222 metabolites with FDR < 0.05 (Figure 3C), ultimately leading to the identification of 126 DEMs. By VIP and *t*-test, a heatmap showed metabolite levels were significantly different between the EC and YMAC groups (top 25) (Figure 3D). The top 15 DEMs (based on VIP) are shown in sTable 3.

ET Group vs YMAT Group

This comparison aims to directly characterize differences in the plasma metabolome between elderly and young adult trauma patients, thereby facilitating the identification of age-specific traumatic response signatures across different age groups. 2D PLS-DA score plot based on the abundance profile of the 309 metabolites demonstrated distinct separation between the ET and YMAT groups (Figure 4A), and cross-validation of PLS-DA revealed that the model exhibited moderate predictive power but relatively high classification accuracy (Figure 4A). PLS-DA identified 121 metabolites with VIP score > 1, the top 15 metabolites based on VIP score were shown (Figure 4B). *T*-test identified 169 metabolites with FDR < 0.05 (Figure 4C), ultimately leading to the identification of 121 DEMs. By VIP and *t*-test, a heatmap showed metabolite levels were significantly different between the ET and YMAT groups (top 25) (Figure 4D). The top 15 DEMs (based on VIP) are shown in sTable 4.

Pathway Analysis

Through pathway analysis of DEMs in ET group vs EC group, we identified 8 SMPs (Figure 5A). Similarly, pathway analysis of YMET group vs YMAC group identified 10 SMPs (Figure 5B). Additionally, comparison of EC group and YMAC group yielded 3 age-related SMPs (Figure 5C), while ET group vs YMAT group revealed 7 SMPs (Figure 5D).

Screening for Unique Metabolic Pathways in Geriatric Trauma

To characterize metabolic pathways specific to geriatric trauma, we generated an IVD integrating 8 SMPs from ET group vs EC group and 10 SMPs from the YMAT group vs YMAC group. Three pathways were uniquely associated with geriatric trauma: galactose metabolism, glyoxylate and dicarboxylate metabolism, and pantothenate and CoA biosynthesis (Figure 6A). Given that these alterations may reflect both geriatric trauma and natural aging, we constructed the second IVD to compare these pathways with SMPs from the EC group vs YMAC group, isolating two metabolic pathways specific to geriatric trauma independent of natural aging: glyoxylate and dicarboxylate metabolism, and

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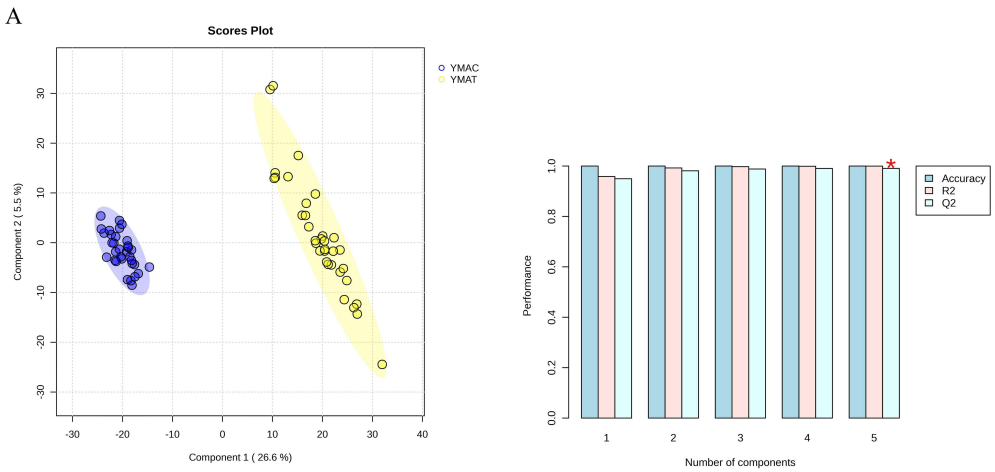
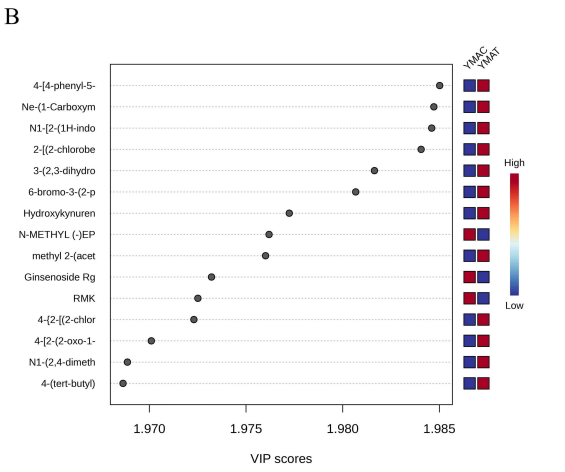
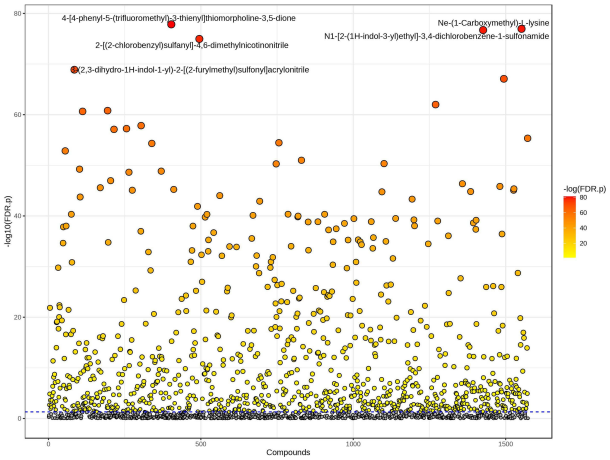


PLS-DA cross validation details:					
Measure	1 comps	2 comps	3 comps	4 comps	5 comps
Accuracy	1.0	1.0	1.0	1.0	1.0
R^2	0.95856	0.99242	0.99754	0.99915	0.99969
Q^2	0.949	0.97972	0.98683	0.98967	0.99013





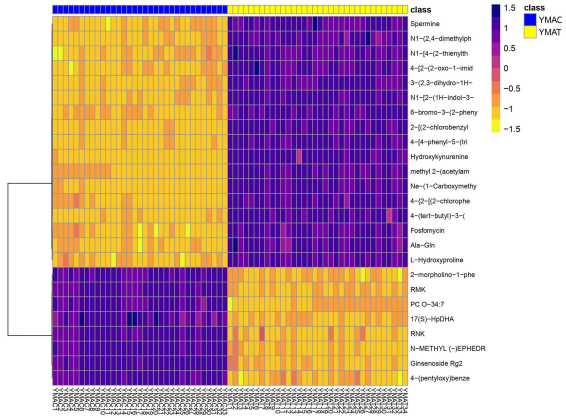
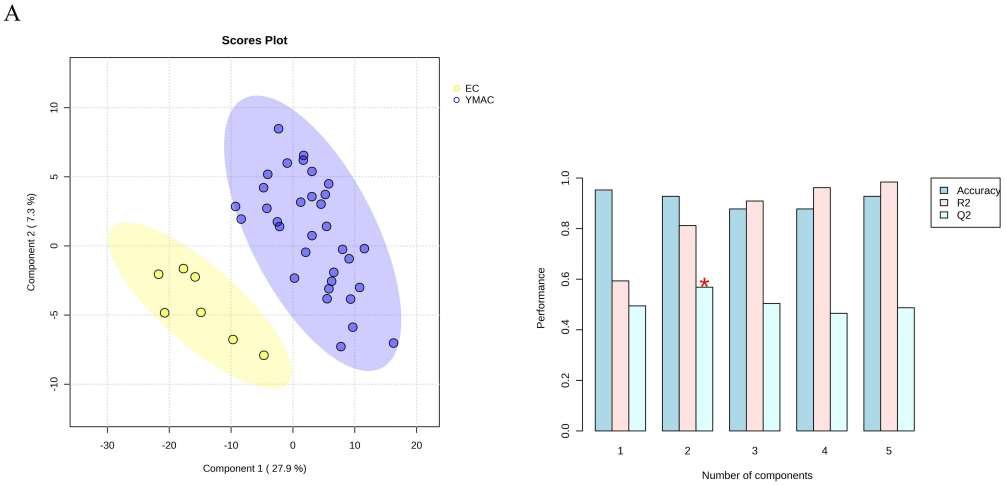
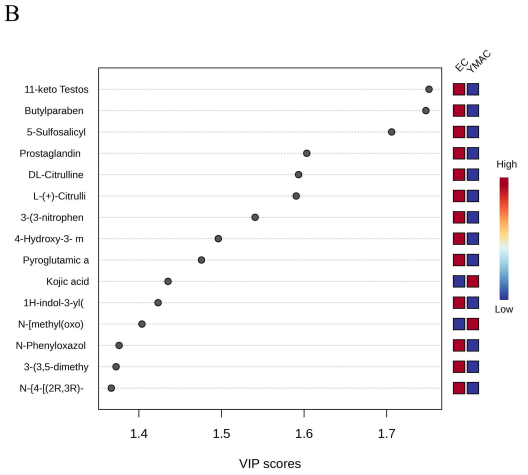


Figure 2 Screening for DEMs Between the YMAT and YMAC groups. (**A**) PLS-DA; (**B**) The top 15 metabolites discriminating the two groups (based on VIP score); (**C**) *t*-test; (**D**) Hierarchical heatmap for top-25 discriminating metabolites. ★ selected principal component based on the largest Q^2.

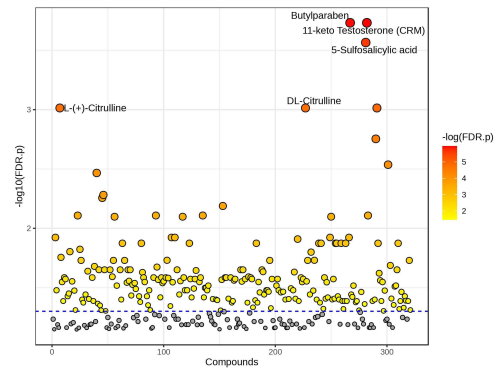
Abbreviations: YMAT, young and middle-aged trauma; YMAC, young and middle-aged control; PLS-DA, partial least-squares discriminant analysis; VIP, variable importance in projection; FDR, false discovery rate.





PLS-DA cross validation details:

Measure	1 comps	2 comps	3 comps	4 comps	5 comps
Accuracy	0.95278	0.92778	0.87778	0.87778	0.92778
R^2	0.59317	0.81195	0.90911	0.9619	0.98421
Q^2	0.50232	0.55383	0.51022	0.43049	0.44208



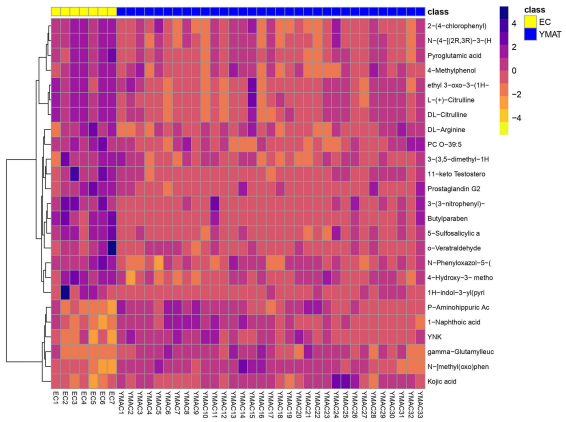


Figure 3 Screening for DEMs Between the EC and YMAC groups. (**A**) PLS-DA; (**B**) The top 15 metabolites discriminating the two groups (based on VIP score); (**C**) *t*-test; (**D**) Hierarchical heatmap for top-25 discriminating metabolites. ★ selected principal component based on the largest Q^2.

Abbreviations: EC, elderly control; YMAC, young and middle-aged control; PLS-DA, partial least-squares discriminant analysis; VIP, variable importance in projection; FDR, false discovery rate.

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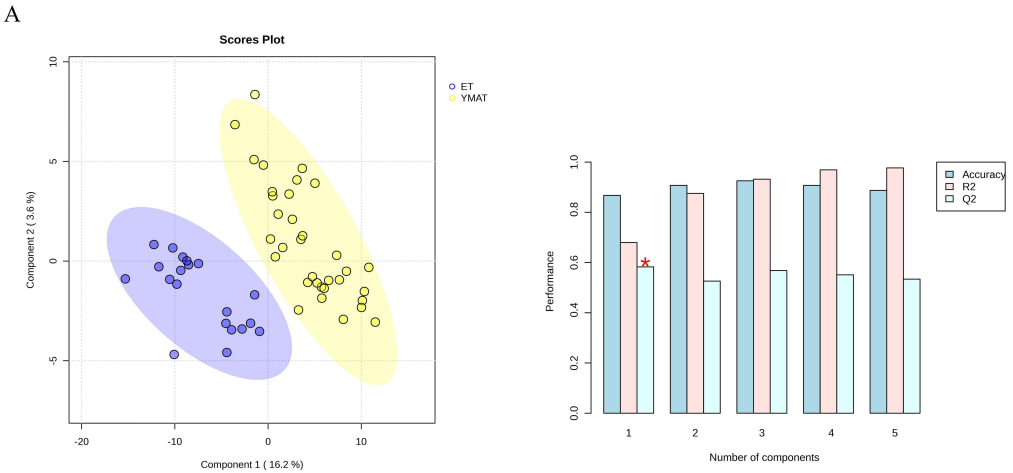
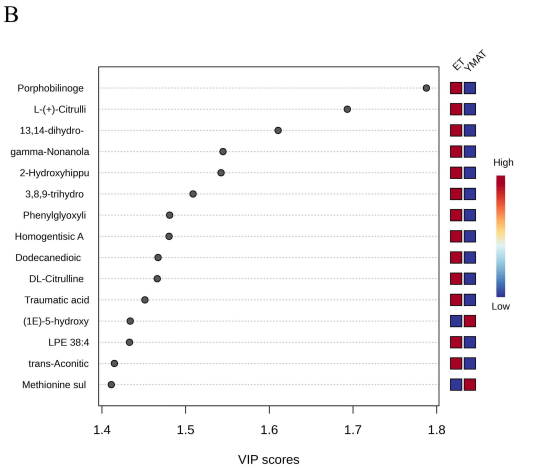
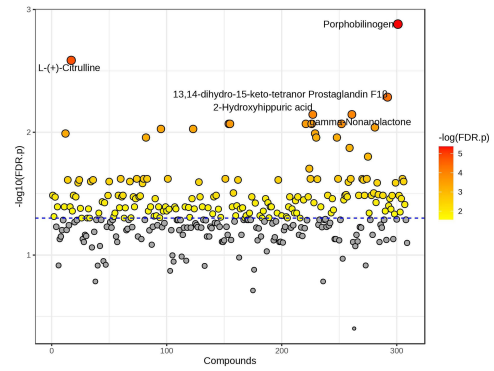


PLS-DA cross validation details:					
Measure	1 comps	2 comps	3 comps	4 comps	5 comps
Accuracy	0.86727	0.90727	0.92545	0.90727	0.88727
R^2	0.67977	0.87532	0.93191	0.96908	0.97683
Q^2	0.5722	0.55293	0.54028	0.56004	0.51561





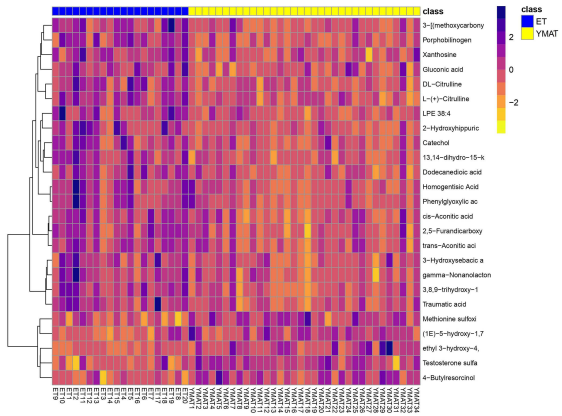


Figure 4 Screening for DEMs Between the ET and YMAT groups. (**A**) PLS-DA; (**B**) The top 15 metabolites discriminating the two groups (based on VIP score); (**C**) *t*-test; (**D**) Hierarchical heatmap for top-25 discriminating metabolites. ★ selected principal component based on the largest Q^2.

Abbreviations: ET, elderly trauma; YMAT, young and middle-aged trauma; PLS-DA, partial least-squares discriminant analysis; VIP, variable importance in projection; FDR, false discovery rate.

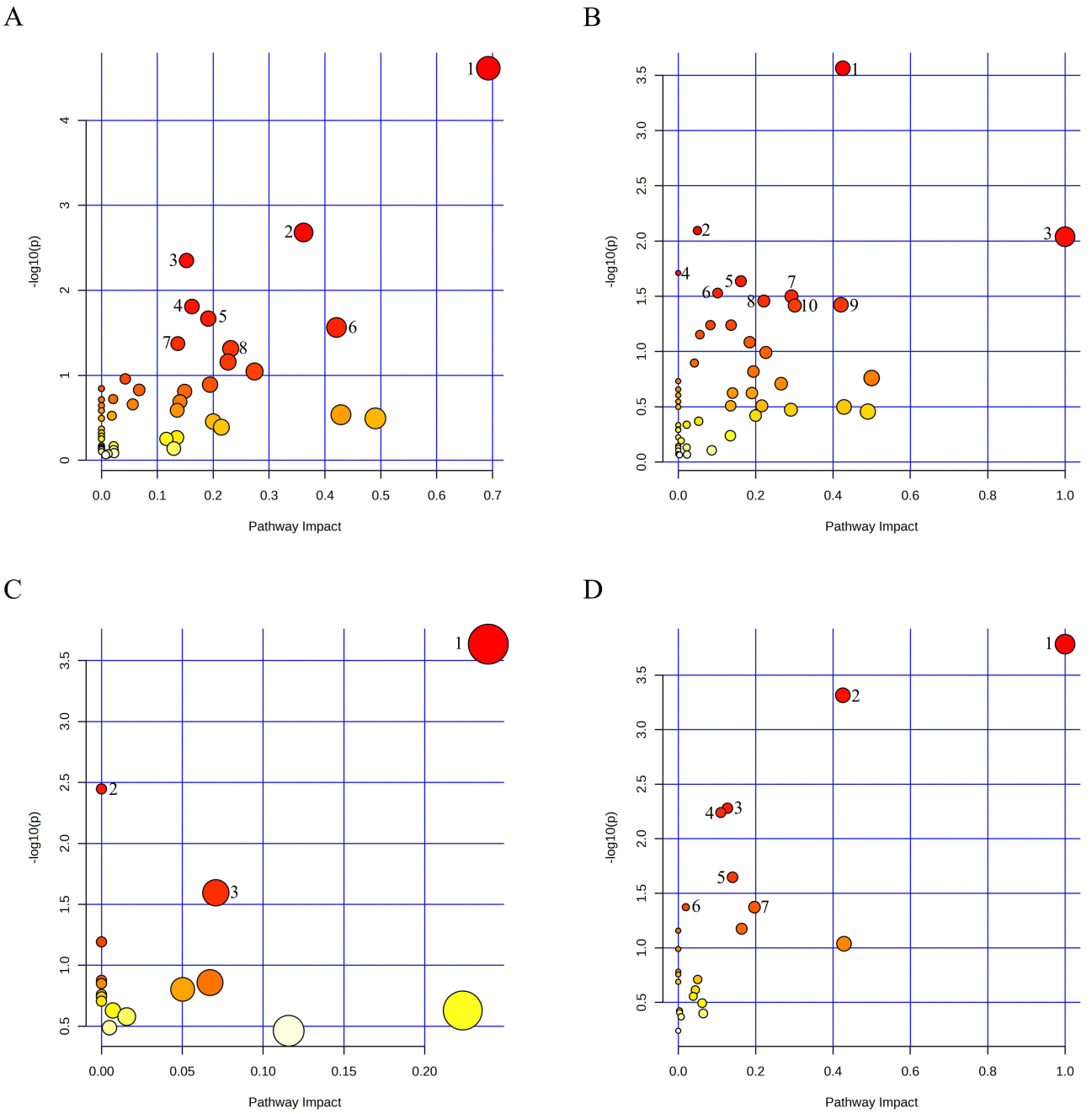


Figure 5 Pathway analysis. (**A**) ET group vs EC group: 1 Caffeine metabolism, 2 Arginine biosynthesis, 3 Galactose metabolism, 4 Glycerophospholipid metabolism, 5 Pyrimidine metabolism, 6 Alanine, aspartate and glutamate metabolism, 7 Glyoxylate and dicarboxylate metabolism, 8 Pantothenate and CoA biosynthesis. (**B**) YMAT group vs YMAC group: 1 Arginine biosynthesis, 2 Glutathione metabolism, 3 Caffeine metabolism, 4 Linoleic acid metabolism, 5 Glycerophospholipid metabolism, 6 One carbon pool by folate, 7 Pyrimidine metabolism, 8 Histidine metabolism, 9 Alanine, aspartate and glutamate metabolism, 10 Tryptophan metabolism. (**C**) EC group vs YMAC group: 1 Arginine biosynthesis, 2 Caffeine metabolism, 3 Galactose metabolism. (**D**) ET group vs YMAT group: 1 Caffeine metabolism, 2 Arginine biosynthesis, 3 Porphyrin metabolism, 4 Glyoxylate and dicarboxylate metabolism, 5 Citrate cycle (TCA cycle), 6 Glutathione metabolism, 7 Alanine, aspartate and glutamate metabolism.
Abbreviations: ET, elderly trauma; EC, elderly control; YMAT, young and middle-aged trauma; YMAC, young and middle-aged control; CoA, coenzyme A.

pantothenate and CoA biosynthesis (Figure 6B). Finally, to validate their specificity for geriatric trauma, the third IVD was generated to intersect SMPs from the ET group vs YMAT group with the one candidate pathways, identifying overlapping pathways as unique signatures of geriatric trauma: glyoxylate and dicarboxylate metabolism (Figure 6C). In the glyoxylate and dicarboxylate metabolism pathway identified from the ET group vs EC group, l-serine and cis-aconitate were significantly altered. The level of l-serine was significantly lower in the ET group, whereas the level of

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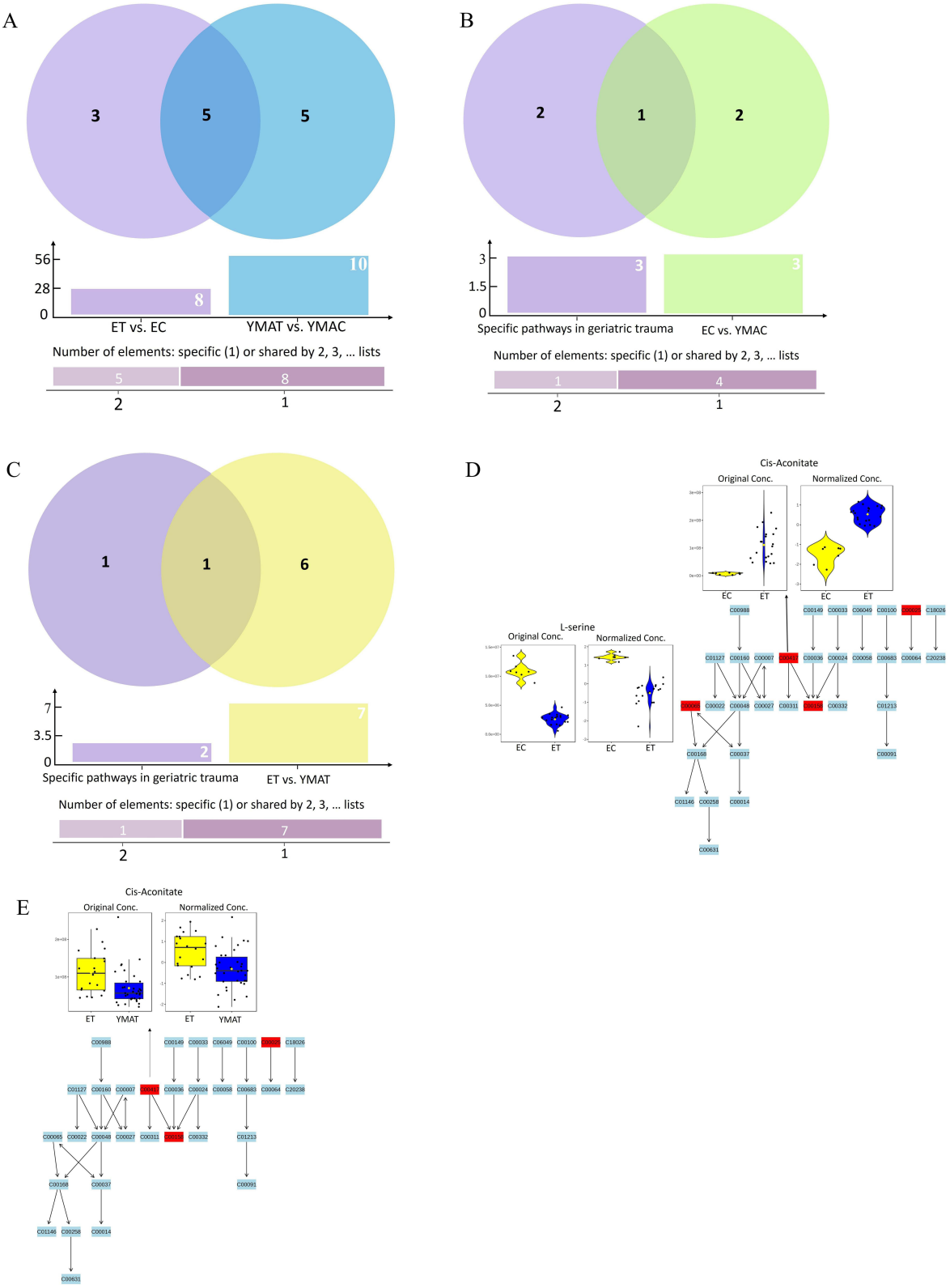

Figure 6 Screening for Unique Metabolic Pathways in Geriatric Trauma. (**A**) Preliminary screening of specific metabolic pathways for geriatric trauma. (**B**) Metabolic pathways specific to geriatric trauma independent of aging. (**C**) Validate the specificity of pathways specific to geriatric trauma. (**D**) Glyoxylate and dicarboxylate metabolism pathway identified from the ET group vs EC group. (**E**) Glyoxylate and dicarboxylate metabolism pathway identified from the ET group vs YMAT group.
Abbreviations: ET, elderly trauma; EC, elderly control; YMAT, young and middle-aged trauma; YMAC, young and middle-aged control.

cis-aconitate was significantly higher (Figure 6D). In the same pathway identified from the ET vs YMAT comparison, only cis-aconitate was significantly altered, and its level was significantly higher in the ET group than in the YMAT group (Figure 6E).

Discussion

This study employed a four-group comparative design (ET vs EC, YMAT vs YMAC, EC vs YMAC, ET vs YMAT), integrating three-step hierarchical screening via Venn diagram analysis to systematically isolate core metabolic pathways. This approach identified trauma-specific pathways in geriatric patients by explicitly correcting for age-related confounders through inter-group control comparisons (EC vs YMAC). By innovatively combining multi-group differential data, the design mitigated limitations of single-group analyses and established a reproducible methodological framework for metabolomics studies of complex diseases with age-dependent pathologies. The identification of aberrant "glyoxylate and dicarboxylate metabolism" unique to geriatric trauma patients offers a novel mechanistic perspective on trauma pathophysiology in the elderly. Elizabeth et al[18] conducted a similar study, noting that differences between trauma patients aged 21–40 and those aged 65+ were not readily apparent. This may be related to differences in injury severity among enrolled trauma patients. In their study, the ISS for the 21–40 trauma group was 18 (14–22), and 16 (13–22) for the 65+ trauma group. To control for bias caused by ISS, our study only enrolled trauma patients with an ISS score of ≥ 16.

In our study, cis-aconitate levels in geriatric trauma patients were significantly higher than those in elderly healthy controls and young-middle aged trauma patients. Cis-aconitate, an intermediate of TCA cycle and glyoxylate and dicarboxylate metabolism, is converted to itaconate by cis-aconitate decarboxylase to regulate inflammatory responses.[19–21] Itaconate and its derivatives exhibit anti-inflammatory activities in preclinical models of sepsis, viral infections, and psoriasis, suggesting potential itaconate-based therapeutic strategies for treating diverse inflammatory diseases.[22–24] Yannick Cyr et al[25] found that itaconate attenuates proinflammatory phospho-signaling and mediates anti-inflammatory rewiring of macrophage populations to alleviate cholesterol-induced inflammation and atherosclerosis. We also found that TCA cycle was significantly altered in the comparison between the ET group and the YMAT group (Figure 5D). TCA cycle is a central pathway of mitochondrial energy metabolism, responsible for converting glucose, fatty acids, and amino acids into adenosine triphosphate.[22] In elderly patients, aging-related decline in mitochondrial function, combined with severe trauma-induced ischemia, hypoxia, and reactive oxygen species production, further impairs mitochondrial function, leading to mitochondrial dysfunction.[26,27] Elevated cis-aconitate levels in geriatric trauma patients may reflect impeded TCA cycle and glyoxylate and dicarboxylate metabolism, leading to accumulation of intermediate metabolites.

Glyoxylate and dicarboxylate metabolism is a biochemical metabolic pathway that encompasses the biosynthesis and catabolism of a range of dicarboxylic acids and related compounds.[28] Glyoxylate and dicarboxylate metabolism, along with TCA cycle and oxidative phosphorylation, are all involved in energy metabolism.[29] Xuechun Yang et al[29] conducted quantitative proteomics and Kbhb PTM proteomics on the hearts of aged mice, revealing that Kbhb-modified proteins are predominantly localized to mitochondrial energy metabolism pathways. Cardiac energy metabolism dysfunction is also one of the important pathogenic mechanisms of atrial fibrillation. Li-Li Zhang et al[30] prospectively collected plasma samples from patients with atrial fibrillation and coronary angiography-negative sinus rhythm patients for metabolomics analysis, identifying significantly enriched pathways in unsaturated fatty acid biosynthesis, glyoxylate and dicarboxylate metabolism, and carbon metabolism. Glyoxylate and dicarboxylate metabolism is downregulated in geriatric trauma patients compared to elderly healthy controls and young-middle aged trauma patients, which may lead to energy metabolism dysfunction in this population. Following trauma, patients exhibit significantly increased energy requirements, and inadequate energy metabolism may lead to slow wound healing, prolonged recovery, and poor prognosis in geriatric trauma patients.

Risk stratification for ET patients is one of key focuses in clinical practice. Though models such as the geriatric trauma outcome score (GTOS), frailty index, and frailty phenotype show promise in predicting outcomes of ET, there remains no ideal risk stratification indicator or model for this patient population.[31] Syam Ravindranath et al[32] found that in ET, GTOS has better predictive performance for mortality compared with age (0.838 vs 0.603) and ISS (0.838 vs 0.799). However, in the intensive care unit, the GTOS demonstrated suboptimal performance (AUC = 0.683) in predicting mortality among ET.[32] This study included trauma patients with ISS ≥ 16. It is possible that cis-aconitate may exhibit better performance in risk stratification among critically ill elderly trauma patients. In the future, plasma cis-aconitate levels in ET could be monitored to explore the

relationship between cis-aconitate and the prognosis of ET. If cis-aconitate is identified as an independent risk factor for mortality in ET, a cut-off value can be calculated. Patients can then be grouped based on this cut-off value, and the prognosis of ET patients in the high-expression and low-expression cis-aconitate groups can be observed prospectively. Furthermore, the value of combining it with traditional disease severity scores (such as GTOS, sequential organ failure assessment, and acute physiology and chronic health evaluation II) for risk stratification in elderly trauma patients could also be explored. This would provide a novel biomarker for risk stratification in elderly trauma patients.

We have noted that ET exhibit a higher prevalence of diabetes and hypertension compared to YMAT. Since both conditions significantly influence systemic metabolism, this observation raises concerns about whether our findings may reflect metabolic disparities attributable to diabetes and hypertension rather than characteristic alterations specific to geriatric trauma itself. Wei Chen et al[33] revealed that insulin resistance may contribute to the development of gestational diabetes mellitus through its interference with the glyoxylate and dicarboxylate metabolism pathway. Linlin Pan et al[34] demonstrated that huanglian decoction ameliorates type 2 diabetes mellitus by interfering with glyoxylate and dicarboxylate metabolism through upregulating the gene and protein expression levels of glucose transporter 4, insulin receptor, and mitogen-activated protein kinase 1. Chenxi Zhang et al[35] found that laminaria japonica fucoidan can indirectly restore the abnormalities of the glyoxylate and dicarboxylate metabolism pathway by regulating the structure of gut microbiota, and ultimately exert an anti-diabetic effect. Other studies have also found that alterations in glyoxylate and dicarboxylate metabolism occur in hypertension.[36,37] These studies suggest that the alterations in glyoxylate and dicarboxylate metabolism in ET may be influenced by diabetes and hypertension. However, we need to note that comorbidities and frailty are the features of the elderly population. It is precisely on this basis that the elderly are more vulnerable, leading to more complex treatment and poorer prognosis when they suffer trauma. The glyoxylate and dicarboxylate metabolism revealed in this study may exactly explain the intrinsic mechanism at the metabolic level of how comorbidities and frailty make ET more vulnerable. These coexisting diseases may not be "noise" that interferes with our conclusions, but an indispensable part of forming the "ET-specific metabolic phenotype". However, this is only a hypothesis synthesized from the literature and the results of this study. In the future, we still need to conduct more refined research: 1) After matching for diabetes and hypertension, explore whether glyoxylate and dicarboxylate metabolism still exhibits significant alterations in elderly trauma patients compared with young and middle-aged adult trauma patients; 2) Stratify elderly trauma patients into subgroups based on the presence or absence of comorbid hypertension and diabetes, and investigate whether there are significant differences in glyoxylate and dicarboxylate metabolism between these two subgroups.

However, this study has several limitations. First, this study is a retrospective investigation with a small sample size, particularly the EC group comprised only 7 subjects. The limited sample size may result in insufficient statistical power and an increased risk of Type II errors. The stability and generalizability of the identified DEMs and associated pathways may be constrained by the restricted cohort. Therefore, our findings and conclusions should be regarded as a preliminary exploration of characteristic metabolic features in ET. Moreover, the retrospective nature of the study precludes prospective control of biases. Thus, further validation in prospective, multi-center, large-sample, independent cohorts is necessary before clinical translation can be considered. Second, the EC group had a gender ratio imbalance; although the difference was not statistically significant ($P > 0.05$), this may have introduced gender-related metabolic bias. Third, being a cross-sectional study, it lacked longitudinal data and thus could not capture the dynamic metabolic changes after trauma. Future longitudinal studies will be required to observe the trajectories of metabolites recovering or delayed metabolic alterations. Fourth, key metabolites in the core pathways were only identified at the "differential expression" level, without cellular or animal experiments to validate their specific regulatory effects on mitochondrial function and inflammatory signaling. Despite these shortcomings, this study still holds great significance. It demonstrates the unique advantages of metabolomics in revealing disease-specific mechanisms in geriatric Trauma, laying the foundation for future large-scale cohort studies, mechanistic validation experiments, and interventional clinical trials.

Conclusion

This study was one of few metabolomics studies that distinguish between geriatric trauma-related metabolic changes and baseline aging factors, and revealed glyoxylate and dicarboxylate metabolism disorder as a key pathway specific to geriatric trauma. Building on this study in the future, we can explore the value of these metabolites in risk stratification

and prognosis assessment of geriatric trauma, as well as the role of correcting glyoxylate and dicarboxylate metabolism in the development of personalized and precision treatment. However, due to the small sample size (especially in the elderly control group), the results of this study are essentially exploratory in nature and require validation of these metabolic markers through larger-scale prospective or longitudinal studies.

Abbreviations

ET, elderly trauma; YMAT, young and middle-aged trauma; EC, elderly control; YMAC, young and middle aged control; DEMs, differentially expressed metabolites; ISS, injury severity score; GCS, Glasgow Coma Scale; WBC, white blood cell count; ANC, absolute neutrophil count; HGB, Hemoglobin; PLT, platelet; Alb, albumin; FIB, fibrinogen; LAC, lactic acid; PLS-DA, Partial least-squares discriminant analysis; VIP, variable importance in projection; FDR, false discovery rate; IVD, interactive venn diagram; SMPs, significantly metabolic pathways; TCA, tricarboxylic acid.

Data Sharing Statement

Data are available upon request from Ke Feng (Email: fengkedoct@163.com).

Ethics Statement

This study adhered to the ethical standards outlined in the Helsinki Declaration of 1964 and its later amendments. The study protocol was approved by the Medical Ethics Committee of General Hospital of Ningxia Medical University (KYLL-2025-1113). Given the retrospective nature of the study and its use of existing metabolomics and proteomics data, the Medical Ethics Committee waived the requirement for informed consent.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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