LC-MS-Based Serum Metabolomics Study of Differential Features Between Mild and Severe Acute Pancreatitis Postprint

Authors: Huang Xiangping, Wu Ling, Tan Chaochao

Date: 2022-07-09T00:00:00+00:00

Abstract

Objective: To investigate differential metabolites between mild acute pancreatitis and severe acute pancreatitis, and to provide novel insights into the pathogenesis and treatment of the transition from mild to severe acute pancreatitis. Methods: Serum samples from 68 patients diagnosed with acute pancreatitis during hospitalization at Hunan Provincial People' s Hospital from August 2020 to March 2021 were collected. According to RAC classification, they were divided into mild acute pancreatitis (40 cases) and severe acute pancreatitis (28 cases). LC-MS metabolomics was used to analyze differential metabolites and metabolic pathways between the two groups. Results: PCA and PLS-DA analyses revealed significant differences in metabolic profiles between mild acute pancreatitis and severe acute pancreatitis. Using criteria of VIP>1, FC>1.5, and P<0.05, 50 differential metabolites and 6 metabolic pathways were screened between the two groups, among which taurine and hypotaurine metabolism and terpenoid backbone biosynthesis were the two most influential metabolic pathways. ROC curve analysis identified 8 differential metabolites with ROC>0.9, namely: 2-phenyl-1,3-propanediol monocarbamate, diphenhydramine N-glucuronide, rac-5,6-epoxy-retinoyl-β-D-glucuronide, hexafluoroisopropanol, NNAL-N-glucuronide, erythritol tetranitrate, 3-hydroxybutyric acid, and tetrahydrodeoxycorticosterone. Among these, the metabolites with elevated expression in severe pancreatitis patients were: 2-phenyl-1,3-propanediol monocarbamate, rac-5,6-epoxy-retinoyl-β-D-glucuronide, hexafluoroisopropanol, erythritol tetranitrate, 3-hydroxybutyric acid, and tetrahydrodeoxycorticosterone; while those with downregulated expression were: diphenhydramine N-glucuronide and NNAL-N-glucoside. Conclusion: Metabolites are significantly altered between mild acute pancreatitis and severe acute pancreatitis, among which 2-phenyl-1,3-propanediol monocarbamate, diphenhydramine N-glucuronide, rac-5,6-epoxy-retinoyl-β-D-glucuronide, hexafluoroisopropanol, NNAL-N-glucuronide, erythritol

tetranitrate, 3-hydroxybutyric acid, and tetrahydrodeoxycorticosterone may serve as potential biomarkers between the two.

Full Text

Serum Metabolomic Study on Differential Profiles Between Mild and Severe Acute Pancreatitis Based on LC-MS

Huang Xiangping[1], Wu Ling[1], Tan Chaochao[1] , Lu Wanying[2]
[1]Department of Laboratory Medicine, Hunan Provincial People' s Hospital, The First Affiliated Hospital of Hunan Normal University, Changsha 410002, Hunan, China
[2]Tongji Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, China

[**Funding**] This research was supported by the Natural Science Foundation of Hunan Provincial Department of Science and Technology (2020JJ8086), the Clinical Guidance Project of Hunan Provincial Department of Science and Technology (2020SK50927), and the Hunan Provincial Department of Education (19B352).

[**Author Introduction**] Huang Xiangping (1993-), male, M.S., primarily engaged in basic research on acute pancreatitis and pancreatic cancer. E-mail: 1416781558@qq.com

[**Corresponding Author**] Dr. Tan Chaochao (Corresponding Author), Deputy Chief Technician, Master' s Supervisor, primarily engaged in research on pancreatic diseases. E-mail: tchchwolf@163.com

Abstract

Objective: To investigate differential metabolites between mild acute pancreatitis (MAP) and severe acute pancreatitis (SAP), providing novel insights into the pathogenic mechanisms underlying disease progression and potential therapeutic strategies.

Methods: Sixty-eight serum specimens from patients diagnosed with acute pancreatitis during hospitalization at Hunan Provincial People' s Hospital between August 2020 and March 2021 were collected. According to the RAC classification, patients were divided into MAP (40 cases) and SAP (28 cases) groups. LC-MS-based metabolomics was employed to analyze differential metabolites and metabolic pathways between the two groups.

Results: PCA and PLS-DA analyses revealed significant differences in metabolic profiles between MAP and SAP. Using criteria of VIP $>$ 1, FC $>$ 1.5, and P $<$ 0.05, 50 differential metabolites and 6 metabolic pathways were identified. Taurine and hypotaurine metabolism and terpenoid backbone biosynthesis were the most significantly affected pathways. ROC curve analysis

identified 8 metabolites with AUC > 0.9: 2-phenyl-1,3-propanediol monocarbamate, diphenhydramine N-glucuronide, rac-5,6-epoxy-retinoyl-β-D-glucuronic acid, hexafluoroisopropanol, NNAL-N-glucuronide, erythrityl tetranitrate, 3-hydroxybutyric acid, and tetrahydrodeoxycorticosterone. Among these, 2-phenyl-1,3-propanediol monocarbamate, rac-5,6-epoxy-retinoyl-β-D-glucuronic acid, hexafluoroisopropanol, erythrityl tetranitrate, 3-hydroxybutyric acid, and tetrahydrodeoxycorticosterone were upregulated in SAP patients, while diphenhydramine N-glucuronide and NNAL-N-glucuronide were downregulated.

Conclusion: Significant metabolic alterations occur between MAP and SAP. The eight identified metabolites may serve as potential biomarkers for distinguishing disease severity, offering new avenues for early diagnosis and treatment of severe acute pancreatitis.

[**Keywords**] LC-MS; mild acute pancreatitis; severe acute pancreatitis; metabolomics

Introduction

Acute pancreatitis (AP) is a common acute abdominal condition clinically characterized by persistent severe upper abdominal pain, often accompanied by abdominal distension, nausea, and vomiting. The incidence of AP has been increasing annually in recent years, with approximately 34 cases per 100,000 population [1]. Common etiological factors include gallstones and alcohol consumption, while other causes such as endoscopic retrograde cholangiopancreatography (ERCP), medications, hypertriglyceridemia, infection, and trauma have also been identified. Current understanding of AP pathogenesis encompasses trypsinogen activation, calcium overload, endoplasmic reticulum stress, mitochondrial dysfunction, and impaired autophagy, with abnormal trypsinogen activation considered a critical pathogenic mechanism. Aberrant activation of pancreatic enzymes can digest the pancreas and surrounding organs, triggering local inflammatory responses that may progress to multiple organ failure [2].

According to the 2012 revised Atlanta classification, AP is categorized into mild acute pancreatitis (MAP), moderately severe acute pancreatitis (MSAP), and severe acute pancreatitis (SAP) [3]. MAP is not associated with organ failure or local/systemic complications and typically resolves within two weeks with extremely low mortality. In contrast, SAP is frequently accompanied by persistent organ failure and carries high mortality rates exceeding 30% [4]. While most patients present with MAP that is self-limiting and resolves within one week, approximately 15% of AP patients develop MSAP or SAP upon initial admission [5], characterized by pancreatic or peripancreatic necrosis and/or organ failure, with mortality rates reaching 20–40% [6]. Early identification and diagnosis of patients at risk for progression to severe disease, followed by timely therapeutic intervention, are crucial for improving prognosis and reducing mor-

tality. Current laboratory tests such as C-reactive protein, blood urea nitrogen, and hematocrit correlate with disease severity but lack sufficient accuracy. Various clinical scoring systems, including the Acute Physiology and Chronic Health Evaluation II (APACHE II) and the Bedside Index for Severity in Acute Pancreatitis (BISAP), also have limitations in predicting SAP development [7].

Metabolomics investigates small-molecule metabolites involved in disease processes, offering value beyond traditional biomarker discovery by elucidating abnormal metabolic pathways. This approach enables comprehensive measurement of endogenous metabolites with molecular weights < 1000 Da, providing a powerful tool for understanding alterations in known metabolic pathways and biological functions in physiological and pathological responses [8]. This study employed metabolomics to screen for differential metabolites between MAP and SAP, identify abnormal metabolic pathways, and explore potential biomarkers to inform early diagnosis and treatment of severe acute pancreatitis.

1. Materials and Methods

1.1 Experimental Materials and Instruments

Methanol and acetonitrile (Thermo Fisher Scientific China Co., Ltd.); distilled water (Watsons Group); EP tubes (Huzhou Zhongrui Precision Technology Co., Ltd.); liquid chromatography-mass spectrometry system (Thermo Scientific, USA); liquid mass analyzer (Bruker, USA); G-560E vortex mixer (Thermo Scientific, USA); low-temperature high-speed centrifuge (Guangzhou Jidi Instrument Co., Ltd.); -80°C ultra-low temperature freezer (Shandong Boke Scientific Instrument Co., Ltd.); medical refrigerator (Shandong Boke Scientific Instrument Co., Ltd.); MetaboAnalyst 5.0 (Bruker, USA).

1.2 Study Subjects

This study utilized serum specimens from 40 MAP patients and 28 SAP patients diagnosed at Hunan Provincial People' s Hospital between August 2020 and March 2021.

Diagnostic criteria for acute pancreatitis: (1) Persistent upper abdominal pain; (2) Serum amylase and/or lipase concentrations at least three times the upper limit of normal; (3) Imaging findings consistent with AP. Diagnosis was established when at least two of these three criteria were met [7].

Inclusion criteria for MAP and SAP: (1) Met RAC classification criteria for MAP and SAP; (2) No other severe metabolic or immune diseases. **Exclusion criteria:** Patients with rheumatic immune diseases, tumors, liver or kidney diseases, or other conditions that could cause pancreatitis were excluded.

1.3 Specimen Collection and Processing

1.3.1 Specimen Collection Venous blood was collected from patients at admission during disease onset, and serum was obtained by centrifugation. All patients met AP diagnostic criteria and were classified into MAP or SAP groups according to the RAC classification.

1.3.2 Specimen Processing A 100 L serum sample was mixed with 10 L internal standard (0.3 mg/mL L-2-chlorophenylalanine in methanol) and vortexed for 10 seconds. Then, 300 L protein precipitant (methanol:acetonitrile, 2:1, v/v) was added, vortexed for 1 minute, and sonicated in an ice bath for 10-15 minutes. The mixture was stored at -20°C for 30 minutes, then centrifuged at 13,000 rpm for 15 minutes at 4°C. Finally, 200 L of supernatant was collected for LC-MS analysis.

1.3.3 Quality Control Preparation Quality control (QC) samples were prepared to evaluate instrument stability and system performance. Ten microliters from each of the 68 samples were pooled into an EP tube, vortexed for 10 seconds, and aliquoted into six tubes (100 L each). Subsequent processing followed the same protocol as for test samples.

1.4 HPLC-MS Analysis

HPLC-MS was employed as the metabolite separation and detection platform to investigate serum metabolic differences between MAP and SAP groups. Data were collected in both positive and negative ion modes.

Ultra-high performance liquid chromatography conditions: Acclaim™ RSLC 120-C18 column (100 mm × 2.1 mm) maintained at 40°C; injection volume: 3 L. Mobile phase A: 0.1% formic acid in water (containing 2 mmol/L ammonium formate); mobile phase B: 0.1% acetonitrile in water. Gradient elution: 2% B for 0–2 min, 50% B for 2–12 min, 90% B for 10–30 min, and 98% B for 30–60 min. Flow rate: 400 L/min.

Mass spectrometry conditions: Electrospray ionization (ESI±) source operated in positive and negative ion modes; high-purity nitrogen as sheath and auxiliary gas at 1.2 L/min; mass scan range: 20–1000 m/z; drying gas temperature: 200°C.

1.5 Quality Control Verification

To assess instrument stability and experimental reproducibility, six QC samples were analyzed. All QC samples showed deviations within 2SD, confirming good instrument stability, reproducibility, and reliability of the experimental data for reflecting true metabolic differences between groups.

1.6 Data Analysis

1.6.1 Data Preprocessing Raw data were preprocessed using Metaboscape 3.0 software for peak extraction, denoising, normalization, and export. Processed data were then matched against the HMDB database to obtain metabolite information including retention time, mass-to-charge ratio, and peak intensity.

1.6.2 Statistical Analysis Preprocessed data were imported into MetaboAnalyst 5.0 for analysis. Data were first normalized to achieve normal distribution, eliminating inter-individual differences and external confounding factors to ensure comparability between metabolites. Univariate analysis using volcano plots identified differential metabolites between groups based on t-test ($P < 0.05$) and fold change ($FC > 1.5$). Principal component analysis (PCA) was performed for data overview and outlier detection. Partial least squares-discriminant analysis (PLS-DA) models were constructed for classification, with model reliability evaluated through K-fold cross-validation and 100 permutation tests. Variable importance in projection (VIP) values from PLS-DA were used to screen potential differential metabolites ($VIP > 1.0$). Hierarchical clustering analysis assessed correlations and clustering patterns among metabolites. Finally, differential metabolites were subjected to metabolic pathway analysis.

2. Results

2.2 Univariate Analysis

Univariate statistical analysis combining t-test and fold change (FC) was applied to screen differential metabolites between MAP and SAP using criteria of $P < 0.05$ and $FC > 1.5$. As shown in Figure 1 [Figure 1: see original paper], 41 differential metabolites were identified in positive ion mode and 30 in negative ion mode.

2.3 Multivariate Statistical Analysis

PCA was performed to analyze serum metabolite profiles between the two groups. As illustrated in Figure 2 [Figure 2: see original paper], minimal intra-group variation and significant inter-group differences were observed in both ion modes, indicating that metabolic characteristics could distinguish between MAP and SAP patients, with clear differences in metabolic profiles.

PLS-DA further analyzed serum metabolite profiles between groups, revealing significant inter-group differences in both positive and negative ion modes. K-fold cross-validation yielded R^2 and Q^2 values of 0.949 and 0.745 in positive mode, and 0.967 and 0.795 in negative mode, respectively. Both R^2 and Q^2 exceeded 0.5, indicating good model fit. Permutation testing (100 iterations) produced P-values < 0.1, confirming absence of overfitting.

Based on VIP values > 1.0, 32 differential metabolites were identified in positive ion mode and 19 in negative ion mode. Hierarchical clustering analysis of MAP

and SAP serum metabolomics data demonstrated clear clustering patterns between groups in both ion modes, as shown in Figure 3 [Figure 3: see original paper].

2.4.1 Serum Metabolite Analysis Results

In positive ion mode, 31 differential metabolites were identified, with 18 upregulated: 2,5-dimethylfuran, gentian violet, cysteine sulfinic acid, adrenalin ethanolamide, 2-pyrrolidone, substance P, acetaminophen, LysoPC(20:1(11Z)), denitrogen mustard, chloroethylamine, tetrahydrodeoxycorticosterone, erythrityl tetranitrate, 3-hydroxybutyric acid, hexafluoroisopropanol, niobium, N-acetylornithine, lysine, and deoxycholic acid; and 14 downregulated: proflavine, glucose-6-phosphate, 8-hydroxyviloxazine glucuronide, diaminopimelic acid, S-3,4-dihydroxybutyric acid, ethanolamine, glucosylgalactosyl hydroxylysine, farnesyl pyrophosphate, 3-methyl-2-oxovaleric acid, 3-hydroxybenzoic acid, 5-fluorouridine, pelargonic acid, and sulfacetamide.

In negative ion mode, 19 differential metabolites were identified, with 7 upregulated: phosphonoformic acid, 2-phenyl-1,3-propanediol monocarbamate, betaine, rac-5,6-epoxy-retinoyl-β-D-glucuronide, acetylcholesterol glucuronide, 3-methyl-2-oxovaleric acid, and 4-vinylphenol sulfate; and 12 downregulated: NNAL-N-glucuronide, phloretin 2' -O-glucuronide, sevoflurane, malathion dicarboxylic acid, canrenone, 2-oxoarginine, trifluoroacetic acid, diphenhydramine N-glucuronide, O-desmethyltramadol glucuronide, 4' -O-methyl-(-)-epicatechin-7-O-beta-glucuronide, DG(18:1(11Z)/20:5(5Z,8Z,11Z,14Z,17Z)/0:0), and 4' -O-methyl-(-)-epicatechin-3' -O-beta-glucuronide.

Metabolic pathway analysis of the significant differential metabolites from both ion modes was performed using MetaboAnalyst 5.0. Based on the KEGG database, 19 metabolic pathways were matched, from which 6 pathways were selected using criteria of -log(p) $>$ 0.5 and pathway impact $>$ 0.05: taurine and hypotaurine metabolism, lysine degradation, starch and sucrose metabolism, glycine, serine and threonine metabolism, and terpenoid backbone biosynthesis.

Table 2 lists the selected pathways with relevant information.

Further analysis of the 50 differential metabolites identified those with AUC $>$ 0.9, as shown in Figure 4 [Figure 4: see original paper] and Table 3 .

Table 3 ROC Diagnostic Performance of Selected Differential Metabolites

Differential Metabolite	AUC	Sensitivity	Specificity
2-Phenyl-1,3-propanediol monocarbamate	96.4%	96.4%	92.5%
Diphenhydramine N-glucuronide	92.8%	92.5%	-
rac-5,6-Epoxy-retinoyl-β-D-glucuronide	96.4%	92.8%	92.5%
Hexafluoroisopropanol	92.8%	-	-
NNAL-N-glucuronide	96.4%	87.5%	-
Erythrityl tetranitrate	92.8%	87.5%	-

Differential Metabolite	AUC	Sensitivity	Specificity
3-Hydroxybutyric acid	92.9%	87.5%	-
Tetrahydrodeoxycorticosterone	96.4%	-	-

3. Discussion

This LC-MS-based serum metabolomics study identified 50 differential metabolites and 6 metabolic pathways between MAP and SAP, with 8 metabolites demonstrating high diagnostic efficacy (AUC $>$ 0.9). In SAP patients, 2-phenyl-1,3-propanediol monocarbamate, rac-5,6-epoxy-retinoyl-β-D-glucuronic acid, hexafluoroisopropanol, erythrityl tetranitrate, 3-hydroxybutyric acid, and tetrahydrodeoxycorticosterone were upregulated, while diphenhydramine N-glucuronide and NNAL-N-glucuronic acid were downregulated.

Xu et al. [9] previously identified 12 differential metabolites between MAP patients and healthy controls using UPLC-HRMS, with octanoylcholine showing the best diagnostic performance. Notably, our study did not identify choline-related metabolites as discriminatory between MAP and SAP, suggesting these may not be ideal markers for distinguishing disease severity.

Other studies using GC-MS and random forest algorithms have investigated differential metabolites in biliary AP (BAP), hyperlipidemic AP (HLAP), and alcoholic AP (AAP) [10]. Interestingly, abnormal glycine, serine, and threonine metabolism was also observed in AAP, suggesting that excessive alcohol consumption may drive AP progression to severe disease through this pathway.

GC-MS analysis of AP patients versus healthy controls identified 3-hydroxybutyric acid, phosphate, glycerol, citric acid, D-galactose, D-mannose, D-glucose, palmitic acid, and serotonin as potential diagnostic biomarkers [11]. Our finding of elevated 3-hydroxybutyric acid in SAP is consistent with these reports. Severe AP causes progressive tissue and organ damage, potentially leading to organ failure. In pancreatic acinar cells, this inflammatory response impairs mitochondrial function, reducing ATP production and promoting conversion of acetyl-CoA to ketone bodies (acetoacetate, 3-hydroxybutyric acid, and acetone), thereby elevating 3-hydroxybutyric acid levels in SAP patients [12].

Hypocalcemia is commonly associated with pancreatic necrosis and persistent low calcium levels can lead to multiple organ failure [13]. Numerous studies have investigated calcium' s role in AP pathogenesis, with intracellular calcium overload and mitochondrial damage representing critical pathogenic steps affecting acinar and ductal cell function. High intracellular calcium concentrations impair ATP production, causing extensive necrosis of pancreatic cells, which further releases trypsin and triggers widespread pancreatic inflammation [14]. Kim et al. [15] demonstrated that monocarbamates can regulate intracellular calcium concentration by blocking T-type calcium channels in neurons, preventing excessive calcium accumulation and exerting neuroprotective effects. While the

mechanism in pancreatic cells remains unelucidated, our finding of elevated 2-phenyl-1,3-propanediol monocarbamate in SAP patients warrants further investigation into whether it similarly modulates calcium homeostasis in pancreatic inflammation.

Glucuronic acid is formed by oxidation of the C-6 hydroxyl group of glucose. Through glucuronidation, it generates highly hydrophilic glucuronide conjugates that are excreted via bile or urine [16]. In humans, numerous endogenous substances (e.g., bilirubin), drugs, and xenobiotics (e.g., environmental toxins) are eliminated through glucuronidation, representing an important detoxification mechanism [17]. Among our differential metabolites, rac-5,6-epoxyretinoyl-β-D-glucuronic acid, diphenhydramine N-glucuronic acid, and NNAL-N-glucuronic acid showed good diagnostic performance. Notably, rac-5,6-epoxyretinoyl-β-D-glucuronic acid was elevated in SAP.

NNAL-N-glucuronide is a glucuronide conjugate of 4-(methylnitrosamino)-1-(3-pyridyl)butanone (NNK), a tobacco-specific lung carcinogen. In humans, NNK is metabolized to NNAL-N-glucuronide for urinary excretion [18]; impairment of this pathway increases lung cancer risk. Cytochrome P450 2A6 (CYP2A6) catalyzes NNK metabolism, reducing lung cancer incidence [19], and has also been implicated in pancreatic cancer [20]. The decreased NNAL-N-glucuronic acid expression in SAP patients may impair detoxification of toxic substances like NNK, leading to accumulation and exacerbating damage to lung and pancreatic tissues, thereby worsening clinical severity. Further investigation of this mechanism may provide novel diagnostic and therapeutic strategies for pancreatitis.

In summary, this LC-MS-based study identified 50 differential metabolites and 6 metabolic pathways between MAP and SAP, with 8 metabolites showing high diagnostic potential. These findings provide new insights for future research on AP diagnosis and treatment.

References

[1] PETROV M S, YADAV D. Global epidemiology and holistic prevention of pancreatitis [J]. Nature Reviews Gastroenterology & Hepatology, 2018, 15(3): 175-184.

[2] ZHENG Z, DING Y-X, QU Y-X, et al. A narrative review of acute pancreatitis and its diagnosis, pathogenetic mechanism, and management [J]. Annals of Translational Medicine, 2021, 9(1): 69.

[3] BANKS P A, BOLLEN T L, DERVENIS C, et al. Classification of acute pancreatitis—2012: revision of the Atlanta classification and definitions by international consensus [J]. Gut, 2013, 62(1): 102-111.

[4] YE S, SI C, DENG J, et al. Understanding the Effects of Metabolites on the Gut Microbiome and Severe Acute Pancreatitis [J]. BioMed Research International, 2021, 2021: 1-10.

[5] JIN Z, XU L, WANG X, et al. Risk Factors for Worsening of Acute Pancreatitis in Patients Admitted with Mild Acute Pancreatitis [J]. Medical Science Monitor, 2017, 23: 1026-1032.

[6] BOXHOORN L, VOERMANS R P, BOUWENSE S A, et al. Acute pancreatitis [J]. The Lancet, 2020, 396(10252): 726-734.

[7] 李非, 曹锋. 中国急性胰腺炎诊治指南（2021）[J]. 中国实用外科杂志, 2021, 41(07): 739-746.

[8] RINSCHEN M M, IVANISEVIC J, GIERA M, et al. Identification of bioactive metabolites using activity metabolomics [J]. Nature Reviews Molecular Cell Biology, 2019, 20(6): 353-367.

[9] XU H, ZHANG L, KANG H, et al. Serum Metabonomics of Mild Acute Pancreatitis [J]. Journal of Clinical Laboratory Analysis, 2016, 30(6): 990-998.

[10] HUANG J-H, HE D, CHEN L, et al. GC-MS based metabolomics strategy to distinguish three types of acute pancreatitis [J]. Pancreatology, 2019, 19(5): 630-637.

[11] XIAO H, HUANG J-H, ZHANG X-W, et al. Identification of potential diagnostic biomarkers of acute pancreatitis by serum metabolomic profiles [J]. Pancreatology, 2017, 17(4): 543-549.

[12] MALÉTH J, RAKONCZAY Z, VENGLOVECZ V, et al. Central role of mitochondrial injury in the pathogenesis of acute pancreatitis [J]. Acta Physiologica, 2013, 207(2): 226-235.

[13] 王先令, 朱丽叶, 陶坤林, et al. 红细胞分布宽度/血清钙离子对急性胰腺炎严重程度的早期诊断价值 [J]. 实用医学杂志, 2020, 36(05): 650-654.

[14] MALÉTH J, HEGYI P. Ca^{2+} toxicity and mitochondrial damage in acute pancreatitis: translational overview [J]. Philosophical Transactions of the Royal Society B: Biological Sciences, 2016, 371(1700): 20150425.

[15] KIM D Y, ZHANG F-X, NAKANISHI S T, et al. Carisbamate blockade of T-type voltage-gated calcium channels [J]. Epilepsia, 2017, 58(4): 617-626.

[16] YANG G, GE S, SINGH R, et al. Glucuronidation: driving factors and their impact on glucuronide disposition [J]. Drug Metabolism Reviews, 2017, 49(2): 105-138.

[17] ČVOROVIĆ J, PASSAMONTI S. Membrane Transporters for Bilirubin and Its Conjugates: A Systematic Review [J]. Frontiers in Pharmacology, 2017, 8: 302.

[18] MURPHY S E, VON WEYMARN L B, PARENTEAU M, et al. Influence of UGT2B10 Genotype on Urinary Excretion of 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol-N-glucuronide by African American Smokers [J]. Chemical Research in Toxicology, 2018, 31(3): 168-175.

[19] YUAN J-M, NELSON H H, CARMELLA S G, et al. CYP2A6 genetic polymorphisms and biomarkers of tobacco smoke constituents in relation to risk of lung cancer in the Singapore Chinese Health Study [J]. Carcinogenesis, 2017, 38(4): 411-418.

[20] ROEMER K, HOCEVAR B A, KAMENDULIS L M, et al. Contribution of Environment and Genetics to Pancreatic Cancer Susceptibility [J]. PloS One, 2014, 9(3): e90052.

Note: Figure translations are in progress. See original paper for figures.