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REVIEW

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Mass spectrometry-based metabolomics in recurrent ischemic stroke: pathophysiological insights, treatment resistance, and synergistic therapies

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Abstract

Recurrent ischemic stroke represents a major unmet clinical challenge, contributing significantly to the global burden of neurological disability and mortality. Despite widespread implementation of guideline-recommended secondary prevention strategies—including antiplatelet therapy, lipid management, and blood pressure control—a substantial proportion of stroke survivors experience subsequent ischemic events (GBD 2019 Stroke Collaborators. Global, regional, and national burden of stroke and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. The Lancet Neurology. 2021;20(10):795–820.). This persistent residual risk suggests that current clinical paradigms fail to capture the complex, heterogeneous biological dysregulation driving the recurrent disease state (Hankey in Lancet Neurol 13:178–194, 2014). Ischemic stroke is fundamentally a catastrophic metabolic crisis, involving rapid bioenergetic failure, profound oxidative stress, and prolonged inflammatory cascades (Dirnagl et al. in Trends Neurosci 22:391–397, 1999). Mass spectrometry (MS)-based metabolomics has emerged as a premier technological platform in preclinical and exploratory clinical research, capable of simultaneously quantifying hundreds to thousands of endogenous small-molecule metabolites (Nicholson and Lindon in Nature 455:1054–1056, 2008). By providing a functional readout of cellular phenotypes, MS metabolomics offers a unique window into the dynamic biochemical alterations that precede, accompany, and follow ischemic injury. This review provides a comprehensive synthesis of recent advances in applying MS-based approaches to dissect the pathophysiology of recurrent ischemic stroke. We critically examine strong evidence implicating core metabolic disruptions, including the "sphingolipid rheostat" and blood–brain barrier integrity, the shift toward pro-inflammatory lipid mediators in the inflammation–thrombosis axis, mitochondrial tricarboxylic acid cycle dysfunction, and the complex interplay between gut microbiota-derived metabolites and host vascular health. Furthermore, we explore the emerging field of pharmacometabolomics, detailing how MS profiling is providing mechanistic insights into resistance to standard antiplatelet therapies, particularly involving clopidogrel bioactivation pathways and arachidonic acid shunting in aspirin-treated patients. The potential of metabolomics to elucidate the biological mechanisms of complementary therapies, such as acupuncture, is also reviewed with critical appraisal. Finally, we provide a realistic and sobering assessment of the current translational gap. We highlight that while MS metabolomics offers unparalleled pathophysiological insights, significant technical and validation hurdles—including standardization of protocols, absolute quantification challenges, and the need for large-scale, diverse cohort studies—must be overcome before these metabolic signatures can

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be translated into viable clinical tools for personalized risk stratification and prevention of recurrent stroke (Wishart in Physiol Rev 99:1819–1875, 2019).

Keywords Ischemic stroke recurrence, Metabolomics, Mass spectrometry, Biomarkers, Antiplatelet resistance, Sphingolipids, Gut microbiota, Pharmacometabolomics

Introduction

Ischemic stroke (IS) remains a leading cause of long-term disability and the second leading cause of death globally [6]. While significant strides have been made in acute management through thrombolysis and thrombectomy, the primary challenge in the post-acute phase is preventing recurrence. The risk of recurrent stroke is highest immediately following the initial event, estimated at approximately 11% within the first year and rising to over 25% within 5 years [7, 8]. Recurrent strokes are frequently more devastating than index events, associated with higher mortality rates, greater functional impairment, and increased healthcare costs [9].

Current secondary prevention strategies rely heavily on population-based guidelines targeting modifiable risk factors, such as hypertension, dyslipidemia, diabetes, and atrial fibrillation, alongside mandatory antithrombotic therapy [10]. However, the persistently high rate of recurrence indicates that these "one-size-fits-all" approaches are insufficient for many patients. The underlying pathophysiology driving recurrence is highly heterogeneous, involving complex interplay between chronic vascular inflammation, persistent hypercoagulability, endothelial dysfunction, and specific genetic predispositions affecting drug metabolism. There is an urgent, unmet need to move beyond traditional clinical risk scores and identify novel molecular markers that can reveal underlying disease mechanisms and stratify patients based on their individual biological risk profiles [11] (Fig. 1).

Ischemic stroke is, at its core, a profound metabolic disorder. The sudden cessation of blood flow triggers an immediate failure of cellular bioenergetics due to oxygen and glucose deprivation, initiating a cascade of excitotoxicity, oxidative stress, membrane lipid degradation, and

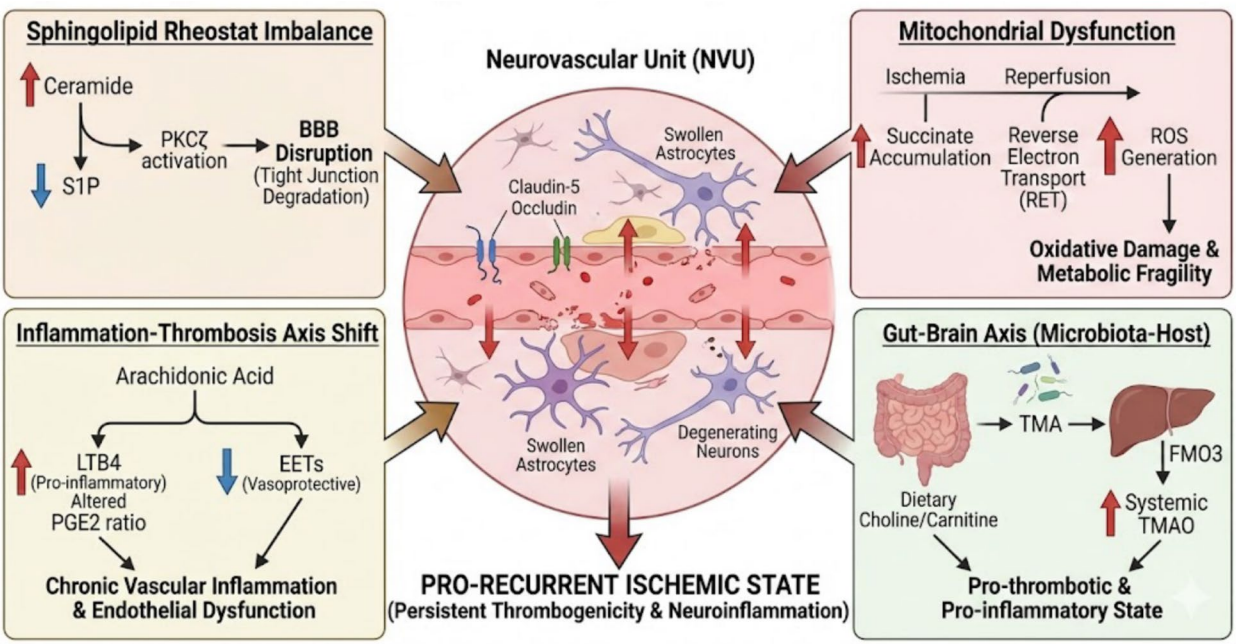

Fig. 1 Integrated pathophysiological pathways. Integrated metabolic pathways driving vulnerability to recurrent ischemic stroke. This schematic illustrates how dysregulation across four core metabolic domains converges to promote a pro-recurrent neurovascular environment. (Top Left) The sphingolipid rheostat shifts toward pro-inflammatory ceramide, activating PKCζ and degrading endothelial tight junctions, compromising blood–brain barrier (BBB) integrity. (Bottom Left) Arachidonic acid metabolism shifts toward pro-inflammatory leukotrienes (e.g., LTB4) and away from vasoprotective epoxyeicosatrienoic acids (EETs). (Top Right) Ischemic accumulation of mitochondrial succinate drives massive reactive oxygen species (ROS) generation upon reperfusion. (Bottom Right) Gut microbiota-derived TMA is converted hepatically to TMAO, promoting systemic vascular inflammation. Together, these pillars create a state of chronic endothelial dysfunction, persistent thrombogenicity, and metabolic fragility

a prolonged neuroinflammatory response that continues long after reperfusion [12]. Metabolomics, the systematic identification and quantification of small-molecule metabolites (< 1500 Da) within biological systems (biofluids, cells, tissues), offers a powerful lens through which to view these processes. As the "omic" layer closest to the clinical phenotype, the metabolome reflects the functional integration of genetic background, transcriptional regulation, protein activity, and environmental exposures (diet, microbiome, medication) [4, 13].

Among metabolomic platforms, mass spectrometry (MS), particularly when coupled with high-resolution separation techniques, such as ultra-performance liquid chromatography (UPLC) or gas chromatography (GC), has become the gold standard for investigating complex diseases like stroke. These platforms offer exceptional sensitivity and dynamic range, capable of detecting metabolites ranging from highly polar energy substrates to complex, hydrophobic lipids (Fig. 2).

While MS-based metabolomics holds immense promise, it is crucial to contextualize its current role. At present, it is primarily a powerful discovery engine in preclinical models and exploratory clinical cohorts, rather than a routine diagnostic tool. This review provides a critical synthesis of how MS-based approaches are reshaping our understanding of the biological drivers of recurrent IS. We move beyond simple biomarker listing to examine key metabolic pathways deeply implicated in recurrence, investigate the metabolic basis of treatment failure, and explore evidence for synergistic therapeutic approaches. Finally, we address the substantial technical and validation hurdles that remain in translating these complex data sets into viable clinical utility (Table 1).

The metabolomic toolbox in stroke research: technologies and challenges

To interpret metabolomic data in stroke research, it is essential to understand the capabilities and limitations of the underlying technologies. MS-based metabolomics is generally divided into two distinct approaches: untargeted discovery and targeted validation [14].

Untargeted vs. targeted approaches

Untargeted Metabolomics is a hypothesis-generating approach aimed at measuring the broadest possible spectrum of metabolites in a sample without prior knowledge of their identity. This typically involves high-resolution mass spectrometry (HRMS) coupled with

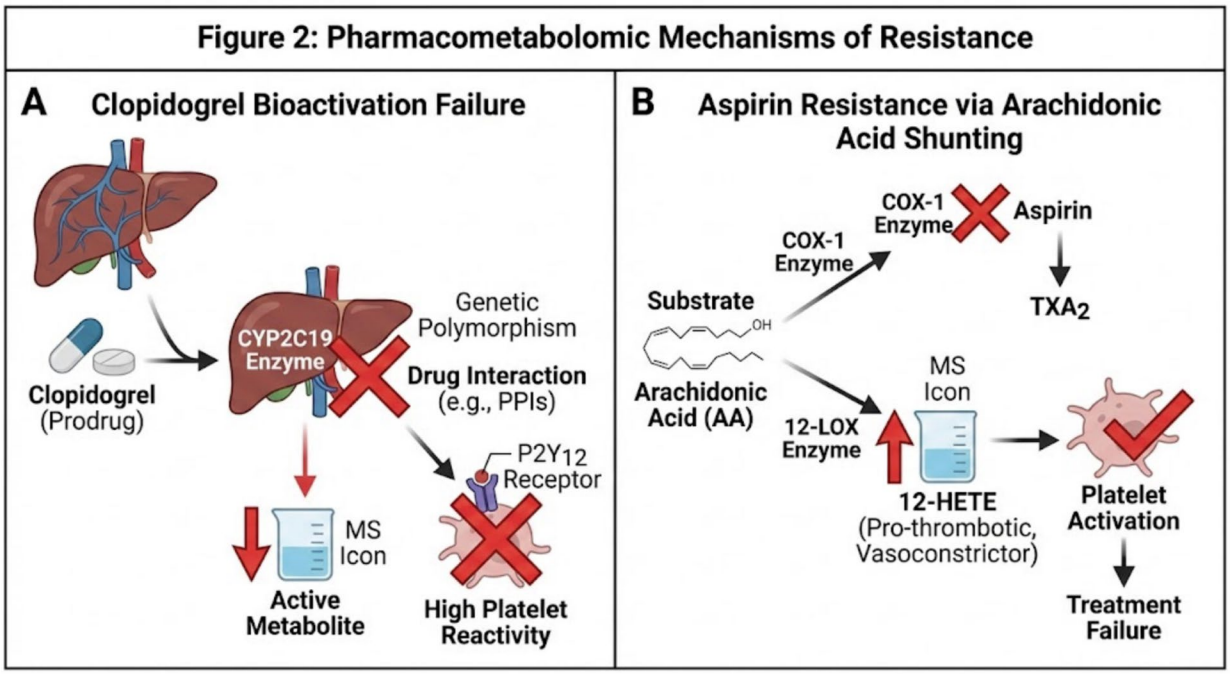

Fig. 2 Pharmacometabolomic mechanisms of resistance. Metabolomic insights into mechanisms of antiplatelet therapy resistance. **A** Clopidogrel is a prodrug requiring hepatic bioactivation, primarily by CYP2C19. Genetic polymorphisms or drug–drug interactions can reduce enzyme activity, leading to insufficient levels of the active metabolite and failure to inhibit the platelet P2Y12 receptor. MS directly measures the active metabolite level, providing a functional readout beyond genotyping. **B** Aspirin inhibits COX-1 to block thromboxane A2 production. However, in some patients, accumulated arachidonic acid substrate is "shunted" down the alternative 12-lipoxygenase (12-LOX) pathway, producing the pro-thrombotic mediator 12-HETE, bypassing the aspirin effect. MS lipidomics can identify elevated 12-HETE as a marker of this specific resistance mechanism

Table 1 Comparison of MS approaches

Feature	Untargeted metabolomics (discovery)	Targeted metabolomics (validation)
Primary goal	Hypothesis generation; Global profiling to identify novel, unexpected metabolic alterations	Hypothesis testing; Precise measurement of pre-defined pathways or specific biomarkers
Metabolite coverage	Broad (hundreds to thousands of features), but many remain chemically unidentified ("unknowns")	Narrow and defined (dozens to hundreds of known, identified compounds)
Quantification	Relative quantification (comparing peak areas between groups). Semi-quantitative at best	Absolute quantification (providing precise concentrations, e.g., μmol/L) using stable isotope internal standards
Sensitivity	Generally lower; optimized for breadth over depth	Highest sensitivity and specificity (especially using triple quadrupole MS in MRM mode)
Key challenge	Metabolite annotation (identification) and complex data processing	Requires prior knowledge of metabolites of interest; limited by the defined scope
Role in stroke research	Discovering new pathways (e.g., initial identification of succinate accumulation or novel gut metabolites)	Validating discovered biomarkers in large cohorts; Pharmacokinetic studies (e.g., measuring drug metabolite levels)

UPLC (LC–MS/MS). In stroke studies, this approach is invaluable for discovering novel pathways. For instance, comparing plasma from recurrent vs. non-recurrent stroke patients might reveal unexpected clusters of dysregulated features [15]. However, a major bottleneck in untargeted analysis is "metabolite annotation"—translating spectral features into definitive chemical identities. Many detected features remain unknowns, limiting biological interpretation (Table 2).

Targeted Metabolomics is a hypothesis-driven approach used to precisely quantify a predefined set of known metabolites. This usually employs triple quadrupole (QqQ) mass spectrometry operating in multiple reaction monitoring (MRM) mode. Targeted assays offer superior sensitivity, specificity, and, crucially, the ability to perform absolute quantification using stable isotope-labeled internal standards [16]. This approach is essential for validating biomarkers discovered in untargeted studies and is the standard for pharmacological studies, such as measuring drug metabolite levels.

Chromatographic separation strategies

The choice of separation technique dictates which part of the metabolome is observed.

Gas chromatography–MS (GC–MS): Requires metabolites to be volatile or derivatized to become volatile. It is excellent for analyzing primary metabolism, including central energy metabolites (TCA cycle intermediates, such as succinate and fumarate), amino acids, and fatty acids. Its spectral libraries are highly standardized, making identification reliable.

Liquid chromatography–MS (LC–MS): The most versatile technique in stroke research. By varying column chemistry (e.g., reversed phase for lipids vs. hydrophilic interaction chromatography [HILIC] for polar compounds), LC–MS can cover a vast range of the metabolome, from complex lipids implicated in inflammation to neurotransmitters involved in excitotoxicity [17].

Critical challenges in stroke metabolomics

Despite its power, applying MS metabolomics to stroke research is fraught with challenges that hinder reproducibility and clinical translation.

Pre-analytical variability: Metabolites are incredibly dynamic. In the context of acute stroke, the time from symptom onset to sample collection is a critical variable that profoundly influences the metabolic profile. Furthermore, variations in sample handling—such as blood collection tube type (EDTA vs. heparin), processing time, time to freezing, and number of freeze–thaw cycles—can artificially alter metabolite levels [18]. For example, bioactive lipids involved in inflammation are rapidly subject to ex vivo oxidation if samples are not properly stabilized.

Biological heterogeneity: Stroke is a heterogeneous syndrome with diverse etiologies (cardioembolic, large artery atherosclerosis, small vessel disease). Lumping these subtypes together in metabolomic studies can obscure subtype-specific metabolic signatures. Furthermore, comorbidities common in stroke populations, such as diabetes and chronic kidney disease, introduce massive metabolic noise that must be carefully controlled for.

The identification vs. quantification trade-off: Untargeted studies provide breadth but often lack precise quantification and definitive identification. Targeted studies provide precision but miss novel compounds outside their defined scope. Bridging this gap remains a major hurdle in moving from discovery to validation.

Table 2 Summary of translational challenges and future directions

Challenge area	Specific issue in stroke metabolomics	Potential solutions and future directions
Validation and reproducibility	Most findings are from small, single-center discovery cohorts. High failure rate in replicating biomarkers across diverse populations	Large-scale, multi-center validation studies with diverse patient demographics and robust external validation cohorts
Standardization	Lack of unified protocols for sample collection (e.g., time-to-freezing), processing, and MS data acquisition leads to high inter-study variability	Development and community-wide adoption of Standard Operating Procedures (SOPs) and use of certified reference materials specifically for stroke biofluids
Clinical workflow integration	High-resolution MS instruments are expensive, slow, and require specialized expertise, making them unfit for acute clinical settings	Development of rapid, high-throughput, simplified targeted assays (e.g., clinical analyzers based on MS) or point-of-care devices for key validated markers
Lack of spatial and temporal context	Bulk plasma/tissue analysis loses spatial information and provides only a static snapshot of dynamic metabolic fluxes	Spatial metabolomics (MALDI–MSI): Mapping metabolite distribution within ischemic tissue structures Stable isotope tracing: Using labeled substrates to measure real-time metabolic pathway activity (in vivo flux)
Biological complexity	Metabolites are downstream of complex gene–environment interactions; single layers of data provide incomplete mechanistic views	Multi-omics integration: Combining metabolomics with genomics, transcriptomics, and proteomics from the same patients to map causal regulatory networks

Core pathophysiological mechanisms of recurrence mapped by MS

MS-based studies have moved beyond identifying single biomarkers to mapping complex, interconnected pathway dysregulations that create a "pro-recurrent" biological milieu.

Dysregulation of sphingolipid metabolism and the neurovascular unit

Sphingolipids are not merely structural components of cell membranes but are potent bioactive signaling molecules regulating cell fate, inflammation, and vascular permeability [19]. MS-based lipidomics has repeatedly identified profound dysregulation of the sphingolipid pathway in both acute stroke and patients at risk for recurrence.

The central axis of this pathway is the balance between ceramide and sphingosine-1-phosphate (S1P), often termed the "sphingolipid rheostat" [20]. Ceramide is generally considered pro-apoptotic and pro-inflammatory, while S1P promotes cell survival, proliferation, and endothelial barrier integrity.

Ceramide and blood–brain barrier (BBB) disruption: In the acute phase of ischemia, MS studies in animal models (e.g., MCAO) and human plasma have consistently shown elevations in various ceramide species [21]. Original mechanistic research indicates that ischemia triggers the activation of sphingomyelinases (enzymes that cleave sphingomyelin to generate ceramide). Elevated intracellular ceramide has been shown to contribute directly to BBB breakdown. Specifically, certain long-chain ceramides can activate signaling cascades, such as the protein kinase C (PKC) pathway, that lead to the disassembly, internalization, and subsequent degradation of critical tight junction proteins, such as claudin-5 and occludin, which maintain the endothelial barrier [22]. This BBB compromise facilitates the infiltration of peripheral leukocytes, exacerbating neuroinflammation and creating a vulnerable neurovascular environment predisposing to secondary injury and potential future events.

The role of S1P: Conversely, circulating S1P, primarily carried by albumin and HDL particles, is crucial for maintaining endothelial barrier function via activation of S1P receptors (S1PR1-3) on endothelial cells. Low plasma levels of S1P in the acute phase have been associated with larger infarct volumes and poorer outcomes [23]. The ratio of plasma ceramide to S1P is emerging as a potential indicator of vascular vulnerability.

Complexity of sphingolipid species: It is critical to note that MS allows for the distinction of specific sphingolipid species based on fatty acyl chain length (e.g., C16:0-ceramide vs. C24:0-ceramide). These species often have distinct biological functions. For instance, some studies suggest C16-ceramide is particularly pro-apoptotic in neurons, while very-long-chain ceramides might have different roles in glial cells or the periphery [24]. This level of molecular granularity, achievable only through MS lipidomics, is essential for understanding the precise pathophysiological roles of these lipids.

The inflammation–thrombosis axis: a lipidomic perspective

Chronic, low-grade inflammation and persistent platelet hyper-reactivity are hallmarks of the "vulnerable patient" at high risk for recurrent stroke. Targeted MS-based lipidomics has been instrumental in profiling the vast array of bioactive lipid mediators, primarily derived from polyunsaturated fatty acids (PUFAs) like arachidonic acid, that orchestrate these processes [25].

The eicosanoid shift: Arachidonic acid is metabolized by three major enzymatic pathways: cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome P450 (CYP) epoxygenases. Under homeostatic conditions, there is a balance between pro-inflammatory/pro-thrombotic mediators (e.g., thromboxane A2, leukotrienes) and anti-inflammatory/pro-resolving mediators (e.g., epoxyeicosatrienoic acids or EETs and lipoxins) [26].

MS analyses of plasma from patients with recent TIA or minor stroke have revealed significant shifts in this balance. Key findings include dysregulation in the leukotriene and prostaglandin pathways involved in acute inflammation. Leukotriene B4 (LTB4), a product of the 5-LOX pathway, is one of the most potent known chemoattractants for neutrophils, driving the early inflammatory response. Prostaglandin E2 (PGE2), a COX product, has complex, context-dependent roles but is central to inflammatory modulation. Clinical research examining cohorts of TIA/minor stroke patients has suggested that an altered balance of pro-inflammatory lipids, such as a high ratio of LTB4 to PGE2, may serve as a prognostic marker reflecting an unstable vascular environment prone to recurrent thrombotic events [27].

Furthermore, CYP-derived EETs are generally regarded as vasodilatory and anti-inflammatory. MS studies have shown that reduced levels of circulating EETs (often due to increased degradation by soluble epoxide hydrolase, sEH) are associated with endothelial dysfunction and increased cardiovascular risk [28]. This has positioned sEH inhibitors as potential therapeutic targets to stabilize the vascular endothelium and prevent recurrence.

Mitochondrial dysfunction and energy metabolism signatures

The brain's disproportionately high energy demand makes it exquisitely sensitive to ischemic disruption. MS metabolomics provides a functional snapshot of

mitochondrial health by quantifying intermediates of the tricarboxylic acid (TCA) cycle and related energy pathways [29].

Acute ischemic signature: Following vessel occlusion, the lack of oxygen halts oxidative phosphorylation. MS studies in MCAO animal models reveal immediate, profound alterations. There is a rapid depletion of ATP and a massive accumulation of its breakdown products (ADP, AMP, adenosine, inosine, hypoxanthine), which spill into the circulation and serve as early markers of tissue energy crisis. Concurrently, anaerobic glycolysis is upregulated, leading to massive lactate accumulation [30].

The succinate reperfusion injury mechanism: Perhaps, the most critical insight from MS metabolomics regarding ischemic injury relates to reperfusion. During ischemia, the TCA cycle stalls, leading to a massive, specific accumulation of the intermediate succinate. Upon reperfusion and the reintroduction of oxygen, this accumulated succinate is rapidly oxidized by succinate dehydrogenase (Complex II of the electron transport chain). This drives extensive reverse electron transport (RET) at mitochondrial complex I, generating a massive burst of reactive oxygen species (ROS) that causes significant reperfusion injury, damaging proteins, lipids, and DNA. This mechanism was elegantly demonstrated using MS-based metabolic profiling in murine models [31].

Chronic mitochondrial impairment and recurrence: Beyond the acute phase, persistent alterations in circulating TCA intermediates indicate chronic mitochondrial dysfunction. Persistent elevations in lactate or alterations in ratios of fumarate/malate in the post-acute phase may reflect ongoing subclinical tissue ischemia or systemic metabolic stress, rendering the brain tissue metabolically fragile and more vulnerable to subsequent insults [32].

Gut microbiota–host metabolic interactions

The gut–brain axis has emerged as a significant modulator of stroke risk and outcome. The gut microbiome functions effectively as a "metabolic organ," processing dietary components into a vast array of bioactive metabolites that enter the host circulation. MS metabolomics is the primary tool utilized to decrypt this complex chemical cross-talk [33].

The TMAO controversy: Attention has focused intensely on trimethylamine N-oxide (TMAO). TMAO is formed via a meta-organismal pathway: gut commensal bacteria express enzymes (such as choline TMA-lyase and CutC/D) that cleave dietary nutrients such as choline, phosphatidylcholine, and L-carnitine to produce trimethylamine (TMA). TMA is absorbed by the host and oxidized in the liver by flavin-containing monooxygenases (primarily FMO3) into TMAO [34].

Early epidemiological studies established a strong association between elevated plasma TMAO levels and major adverse cardiovascular events (MACE) [35]. In the context of stroke, several cohorts have shown that elevated baseline TMAO is associated with poor functional outcome and increased risk of recurrence [36]. However, the precise mechanistic link remains a subject of intense debate and investigation. Initial preclinical studies suggested TMAO might directly enhance platelet hyperreactivity by increasing calcium release from intracellular stores in response to agonists, such as ADP and thrombin [37]. However, subsequent and more recent research has provided conflicting results, with some studies failing to replicate this direct platelet effect. Current consensus is shifting toward more nuanced mechanisms, suggesting chronic TMAO elevation may promote vascular inflammation, induce endothelial dysfunction by reducing nitric oxide bioavailability, or alter cholesterol transport, thereby creating a pro-atherothrombotic environment rather than acting as a direct acute platelet agonist [38, 39].

Beyond TMAO: MS metabolomics is also uncovering other gut-derived metabolites relevant to stroke. Short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, produced by bacterial fermentation of dietary fiber, are generally considered neuroprotective and anti-inflammatory. Reduced levels of SCFA-producing bacteria and corresponding plasma SCFAs have been observed in post-stroke patients [40]. In addition, bacterial metabolism of tryptophan produces various indole derivatives, some of which act as ligands for the aryl hydrocarbon receptor (AhR) and play roles in regulating immune responses and gut barrier integrity, potentially influencing systemic inflammation post-stroke [41].

Pharmacometabolomics: dissecting treatment resistance

A significant proportion of recurrent strokes occur in patients who are already adherent to guideline-recommended antiplatelet therapy (aspirin and/or clopidogrel). This clinical phenomenon is termed "treatment failure" or "high on-treatment platelet reactivity (HTPR)" [42]. MS-based pharmacometabolomics is providing crucial insights into the molecular basis of this resistance, moving beyond simple genetics.

Clopidogrel bioactivation pathway

Clopidogrel is a pro-drug that is inactive in vitro. It requires a two-step hepatic bioactivation process involving several cytochrome P450 (CYP) enzymes to generate its active thiol metabolite, which irreversibly inhibits the platelet P2Y12 ADP receptor.

Beyond CYP2C19 genotyping: It is well-established that loss-of-function polymorphisms in the CYP2C19 gene lead to reduced active metabolite formation and are associated with higher risk of ischemic events [43]. However, genotyping alone explains only a fraction of the variability in clopidogrel response. Many patients with normal genotypes still exhibit HTPR.

Targeted MS-based assays allows for the direct quantification of clopidogrel, its intermediate metabolites, and, crucially, its short-lived active metabolite in patient plasma. This approach provides a functional phenotypic readout that integrates genetic factors with non-genetic variables that genetics cannot capture, such as drug–drug interactions (e.g., proton pump inhibitors inhibiting CYPs), liver function, and age. Studies have demonstrated that low plasma levels of the active metabolite, as measured directly by MS, correlate better with ex vivo platelet function tests and clinical ischemic outcomes than CYP2C19 genotype alone in certain populations [44]. This suggests that therapeutic drug monitoring via MS could potentially guide personalized antiplatelet dosing, though practical implementation remains a challenge.

Aspirin resistance and arachidonic acid shunting

Aspirin exerts its antithrombotic effect by irreversibly acetylating cyclooxygenase-1 (COX-1) in platelets, thereby blocking the conversion of arachidonic acid to the potent platelet activator and vasoconstrictor, thromboxane A2 (TXA2). "Aspirin resistance" is multifactorial, but metabolomics has shed light on alternative metabolic pathways that may bypass COX-1 inhibition [45].

The 12-LOX/12-HETE pathway: MS lipid profiling has revealed that in some patients exhibiting high platelet reactivity despite aspirin therapy, the inhibition of COX-1 may lead to a "shunting" of available arachidonic acid substrate toward other enzymatic pathways. Specifically, arachidonic acid may be metabolized by platelet 12-lipoxygenase (ALOX12) to produce 12-hydroxyeicosatetraenoic acid (12-HETE). 12-HETE is a bioactive lipid known to promote platelet activation, integrin activation, and vasoconstriction, potentially undermining the therapeutic effect of aspirin [46]. Elevated plasma levels of 12-HETE have been observed in aspirin-treated stroke patients with recurrent events compared to those without recurrence, suggesting it may serve as a marker of this metabolic escape pathway and a potential target for novel therapeutic intervention.

Oxidative stress and isoprostanes: Furthermore, under conditions of high oxidative stress—common in vascular disease—arachidonic acid can be oxidized non-enzymatically by free radicals to form F2-isoprostanes. These compounds are structurally similar to prostaglandins and can activate thromboxane receptors, leading to platelet activation and vasoconstriction predominantly independent of COX-1 activity, thus rendering aspirin ineffective against this specific pathway. MS is the gold standard for quantifying these specific markers of in vivo lipid peroxidation [47].

Synergistic therapies: a metabolomic perspective on acupuncture

Integrative medicine approaches, combining traditional therapies like acupuncture with standard pharmacotherapy, are increasingly being explored for stroke rehabilitation and secondary prevention, particularly in Asian countries. Understanding the biological basis of these interventions is crucial for their acceptance and integration. MS metabolomics is being utilized to investigate the mechanisms of electroacupuncture (EA).

Studies using pre-clinical rodent models of MCAO have applied untargeted and targeted MS/MS approaches to examine brain tissue, plasma, and cerebrospinal fluid following EA treatment at specific, traditionally defined acupoints, such as GV20 (Baihui, located on the head) and ST36 (Zusanli, located on the hind limb).

Neurotransmitter modulation: Original research in these models indicates that EA treatment can significantly modulate the balance of excitatory and inhibitory neurotransmitters in ischemic brain regions. Specifically, targeted MS studies have observed decreases in extracellular glutamate levels (thereby reducing excitotoxicity) alongside increases in gamma-aminobutyric acid (GABA) levels in the penumbral cortex and striatum following treatment [48]. This restorative effect on the excitation/inhibition balance is thought to contribute to neuroprotection and improved functional recovery.

Metabolic network effects: Beyond neurotransmitters, metabolomic profiling has suggested that EA may influence broader metabolic networks, including downregulating markers of oxidative stress, modulating inflammatory lipid mediators, and potentially influencing energy metabolism pathways damaged by ischemia.

Critical appraisal of current evidence: While these metabolomic findings in animal models offer intriguing hypotheses regarding the biological mechanisms of acupuncture, it is essential to view the current evidence base with critical caution. Many of these studies are conducted in small animal cohorts with heterogeneous treatment protocols (varying frequency, intensity, and timing of EA). Crucially, high-quality clinical metabolomic studies on acupuncture in human stroke patients are scarce and often lack rigorous sham acupuncture controls, making it difficult to distinguish specific physiological effects from placebo effects or the natural history of recovery [49]. Therefore, while the preclinical metabolic data are suggestive of potential neuroprotective mechanisms,

strong clinical evidence supporting the specific role of acupuncture in preventing stroke recurrence is currently insufficient, and rigorous, large-scale randomized controlled trials with embedded mechanistic biomarkers are needed.

Challenges in clinical translation and future directions

Despite the profound mechanistic insights provided by MS metabolomics into recurrent ischemic stroke, the translation of these discoveries into routine clinical practice faces substantial, and currently insurmountable, hurdles. We are at a stage of high promise but limited practical application.

The validation crisis

The most critical challenge is the lack of large-scale, independent validation. The vast majority of metabolic biomarkers identified for stroke recurrence are derived from single-center discovery cohorts with relatively small sample sizes. These initial findings often fail to replicate in subsequent studies due to differences in patient demographics, genetic backgrounds, dietary habits, and comorbidities [50]. Before any metabolic signature can be considered for clinical use, it must undergo rigorous validation in large, multi-center, diverse patient populations to establish robust clinical utility, define normal reference ranges, and determine decision thresholds.

Standardization and interoperability

Currently, there is a significant lack of standardized protocols across the entire metabolomics workflow. Laboratories differ in their sample collection tubes, processing times, storage conditions, lipid extraction protocols, LC column selection, MS instrument parameters, and data processing software. This leads to high inter-study variability, making it nearly impossible to directly compare quantitative results across different publications or to conduct effective meta-analyses. The development and widespread adoption of standard operating procedures (SOPs) and reference materials for stroke metabolomics is an absolute prerequisite for clinical translation.

Integration with clinical workflow

Current research-grade high-resolution MS instruments are expensive, require highly specialized technical expertise to operate and maintain, and have relatively low throughput compared to standard clinical chemistry analyzers. Turnaround times for complex metabolomic analyses are measured in days or weeks, not hours. For metabolomics to impact acute or sub-acute stroke care decisions, the development of simplified, robust, high-throughput clinical assays—perhaps using targeted triple–quadrupole MS or even developing point-of-care devices for key validated metabolites—is necessary.

Future directions: emerging technologies

The future of this field likely lies in moving beyond static snapshots of plasma to more dynamic and integrated views of tissue metabolism.

Multi-omics integration: Stroke is not caused by genes or metabolites alone but by their complex interaction. Integrating metabolomic data with genomics (GWAS), transcriptomics, and proteomics from the same patients will provide a more holistic view of the causal regulatory networks driving recurrence, potentially identifying upstream drug targets rather than just downstream markers [51].

Spatial metabolomics: Traditional metabolomics requires tissue homogenization, losing all spatial context. Emerging technologies such as matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI–MSI) allow for the mapping of metabolite distributions directly within tissue sections with near-cellular resolution [52]. In preclinical models, this is already being used to delineate the metabolic geography of the ischemic core vs. the salvageable penumbra, revealing spatially distinct lipid and energy signatures that bulk analysis misses.

In vivo metabolic tracing: Static metabolite levels do not show metabolic flux (the rate of turnover). The use of stable isotope-labeled tracers (e.g., 13C-glucose and 13C-succinate) coupled with MS or advanced magnetic resonance spectroscopy (hyperpolarized MRS) allows for the real-time tracking of metabolic pathways in vivo. This approach is crucial for definitively proving hypotheses such as the succinate accumulation/oxidation mechanism in living stroke models [53].

Conclusion

Mass spectrometry-based metabolomics has proven to be an exceptionally powerful discovery engine in stroke research. It has moved the field beyond viewing ischemic stroke solely as a vascular occlusion event, revealing it as a complex systemic condition driven by interconnected metabolic dysregulations involving sphingolipid toxicity, unresolved inflammation, chronic mitochondrial bioenergetic failure, and gut microbiome–host misalignment. Furthermore, it is shedding critical light on the metabolic mechanisms underpinning resistance to standard antiplatelet therapies, suggesting routes for personalized pharmacological management.

However, it is imperative to recognize that the field is currently at a pre-clinical crossroads. While the biological insights are compelling, the gap between identifying a metabolic pathway in a mouse model or a small human cohort and implementing a validated biomarker

test in a stroke clinic remains vast. Moving these findings from the laboratory to the bedside requires a concerted, global effort focused on rigorous, multi-center validation, technological standardization, and the development of practical, rapid clinical tools [54]. Until these challenges are met, MS metabolomics will remain a brilliant research tool rather than a part of routine clinical care for preventing recurrent stroke.

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All authors contributed to the conception, design, and drafting of the manuscript. All authors read and approved the final manuscript.

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No datasets were generated or analysed during the current study.

Declarations

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Consent for publication
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Competing interests
The authors declare no competing interests.

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References

1. GBD 2019 Stroke Collaborators. Global, regional, and national burden of stroke and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet Neurol. 2021;20(10):795–820.
2. Hankey GJ. Secondary stroke prevention. Lancet Neurol. 2014;13(2):178–94.
3. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci. 1999;22(9):391–7.
4. Nicholson JK, Lindon JC. Systems biology: metabonomics. Nature. 2008;455(7216):1054–6.
5. Wishart DS. Metabolomics for investigating physiological and pathophysiological processes. Physiol Rev. 2019;99(4):1819–75.
6. Feigin VL, Brainin M, Norrving B, et al. World Stroke Organization (WSO): Global Stroke Fact Sheet 2022. Int J Stroke. 2022;17(1):18–29.
7. Mohan KM, Wolfe CDA, Rudd AG, Heuschmann PU, Kolominsky-Rabas PL, Grieve AP. Risk and cumulative risk of stroke recurrence: a systematic review and meta-analysis. Stroke. 2011;42(5):1489–94.
8. Amarenco P, Lavallée PC, Monteiro Tavares L, et al. Five-year outcomes in patients with transient ischemic attack or minor ischemic stroke: the TIAregistry. org project. New England J Med. 2018;378(23):2182–90.
9. Hardie K, Hankey GJ, Jamrozik K, Broadhurst RJ, Anderson C. Ten-year risk of first recurrent stroke and disability after first-ever stroke in the Perth Community Stroke Study. Stroke. 2004;35(3):731–5.
10. Kleindorfer DO, Towfighi A, Chaturvedi S, et al. 2021 Guideline for the Prevention of Stroke in Patients With Stroke and Transient Ischemic Attack: A Guideline From the American Heart Association/American Stroke Association. Stroke. 2021;52(7):e364–467.
11. Jickling GC, Sharp FR. Blood biomarkers of ischemic stroke. Neurotherapeutics. 2011;8(3):349–60.
12. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. Nat Med. 2011;17(7):796–808.
13. Psychogios N, Hau DD, Peng J, et al. The human serum metabolome. PLoS ONE. 2011;6(2):e16957.
14. Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL. Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. Chem Soc Rev. 2011;40(1):387–426.
15. Jové M, Mauri-Capdevila G, Suárez I, et al. Metabolomics predicts stroke recurrence after transient ischemic attack or minor stroke. Ann Neurol. 2015;77(1):36–46.
16. Liebeke M, Puchałka J. Mass spectrometry based targeted metabolomics for the analysis of pathways and metabolite fluxes. Mol Biosyst. 2013;9(5):819–27.
17. Theodoridis G, Gika HG, Wilson ID. LC-MS-based methodology for global metabolite profiling in metabonomics/metabolomics. TrAC Trends Anal Chem. 2008;27(3):251–60.
18. Kamlage B, Mansfeld FM, Schluter N, et al. To freeze or not to freeze: Pre-analytical variances in the comprehensive metabolic profiling of human blood plasma and serum based on ultra-high performance liquid chromatography coupled to mass spectrometry. Metabolomics. 2018;14(3):35.
19. Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. Nat Rev Mol Cell Biol. 2008;9(2):139–50.
20. Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease. Nature. 2014;510(7503):58–67.
21. Fick WS, Stutz LC, Knape T, et al. Alterations in the sphingolipidome during the acute phase of ischemic stroke. J Cereb Blood Flow Metab. 2021;41(9):2387–401.
22. Stamatovic SM, Keep RF, Qin ZJ, Andjelkovic AV. Protein kinase C signaling pathways in blood-brain barrier pathology in cerebral ischemia. J Cereb Blood Flow Metab. 2006;26(7):931–43.
23. Sun Y, Han J, Guan Y, et al. Plasma sphingosine-1-phosphate levels are associated with clinical outcome in ischemic stroke patients. Neuroendocrinology. 2020;110(6):486–94.
24. Ben-David O, Futerman AH. The role of the ceramide acyl chain length in neurodegeneration: involvement of ceramide synthases. Neuromolecular Med. 2010;12(4):341–50.
25. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol. 2008;8(5):349–61.
26. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science. 2001;294(5548):1871–5.
27. Cipollone F, Cicolini G, Bucci M. Cyclooxygenase and 5-lipoxygenase coordinating inflammation in the atherosclerotic plaque. Curr Opin Lipidol. 2005;16(5):505–10.
28. Imig JD, Hammock BD. Soluble epoxide hydrolase as a therapeutic target for cardiovascular diseases. Nat Rev Drug Discov. 2009;8(10):794–805.
29. Lodi R, Tonon C, Strata P. Non-invasive in vivo assessment of brain metabolism. Funct Neurol. 2011;26(3):133–6.
30. Hinzman JM, Thomas TC, Quintero JE, et al. Disruptions in the regulation of extracellular glutamate by neurons and glia in the rat striatum two days after diffuse brain injury. J Neurotrauma. 2012;29(6):1197–208.
31. Chouchani ET, Pell VR, Gaude E, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature. 2014;515(7527):431–5.
32. Wang Y, Deng X, Zou J, et al. Metabolomic profiling of plasma abnormalities in patients with acute ischemic stroke. J Proteome Res. 2020;19(10):4057–67.

33. Schroeder BO, Bäckhed F. Signals from the gut microbiota to distant organs in physiology and disease. Nat Med. 2016;22(10):1079–89.
34. Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-N-oxide. MBio. 2015;6(2):e02481.
35. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011;472(7341):57–63.
36. Zhu W, Gregory JC, Org E, et al. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. Cell. 2016;165(1):111–24.
37. Tang WHW, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med. 2013;368(17):1575–84.
38. Roberts AB, Gu X, Buffa JA, et al. Development of a gut microbe-targeted nonlethal therapeutic to inhibit thrombosis potential. Nat Med. 2018;24(9):1407–17.
39. Chen ML, Yi L, Zhang Y, et al. Resveratrol attenuates trimethylamine-N-oxide (TMAO)-induced atherosclerosis by regulating TMAO synthesis and bile acid metabolism via gut microbiota remodeling. EBioMedicine. 2016 7 30–42
40. Sadler R, Cramer JV, Heindl S, et al. Short-chain fatty acids improve poststroke recovery via immunological mechanisms. J Neurosci. 2020;40(5):1162–73.
41. Rothhammer V, Mascanfroni ID, Bunse L, et al. Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. Nat Med. 2016;22(6):586–97.
42. Floyd CN, Ferro A. Mechanisms of antiplatelet drug resistance. Br J Clin Pharmacol. 2014;77(6):1061–9.
43. Mega JL, Close SL, Wiviott SD, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. N Engl J Med. 2009;360(4):354–62.
44. Simon T, Verstuyft C, Mary-Krause M, et al. Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med. 2009;360(4):363–75.
45. Hankey GJ, Eikelboom JW. Aspirin resistance. Lancet. 2006;367(9510):606–17.
46. Yeung J, Apopa PL, Vesci J, et al. 12-HETrE, a 12-lipoxygenase metabolite of dihomo-γ-linolenic acid, inhibits human platelet activation by interfering with multiple signaling pathways. Blood Adv. 2020;4(20):5110–22.
47. Morrow JD, Roberts LJ 2nd. Mass spectrometry of prostanoids: F2-isoprostanes produced by non-cyclooxygenase free radical-catalyzed mechanism. Methods Enzymol. 1994;233:163–74.
48. Liu F, Li ZM, Jiang YJ, Li YQ. Electroacupuncture reduces neuronal apoptosis in hippocampus by suppressing oxidative stress in cerebral ischemia-reperfusion rats. Neural Regen Res. 2015;10(8):1283–8.
49. Yang A, Wu HM, Tang JL, Xu L, Yang M, Liu GJ. Acupuncture for stroke rehabilitation. Cochrane Database Syst Rev. 2016;8:CD004131.
50. Xia J, Broadhurst DI, Wilson ID, et al. Differential metabolomics for quantitative assessment of reproduction in a systems biology framework. Nat Protoc. 2013;8(3):427–39.
51. Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. Genome Biol. 2017;18(1):83.
52. Alexandrov T. Spatial metabolomics and imaging mass spectrometry in the age of biology. Annu Rev Biomed Data Sci. 2020;3:61–87.
53. Lane AN, Fan TWM, Higashi RM. Metabolic flux analysis in mammalian systems. Curr Opin Chem Biol. 2009;13(5–6):568–75.
54. Wishart DS. Emerging applications of metabolomics in drug discovery and precision medicine. Nat Rev Drug Discov. 2016;15(7):473–98.

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