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Lessons from Metabonomics   
on the Neurobiology of Stroke

The Neuroscientist

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Abstract

The application of metabonomic science to interrogate stroke permits the study of metabolite entities, small enough to cross the blood-brain barrier, that provide insight into neuronal dysfunction, and may serve as reservoirs of biomarker discovery. This systematic review examines the applicability of metabolic profiling in ischemic stroke research. Six human studies utilizing metabolic profiling to analyze biofluids from ischemic stroke patients have been included, employing 1H-NMR and/or mass spectrometry to analyze plasma, serum, and/or urine in a targeted or untargeted fashion. Three are diagnostic studies, and one investigates prognostic biomarkers of stroke recurrence following transient ischemic attack. Two studies focus on metabolic distinguishers of depression or cognitive impairment following stroke. Identified biomarkers from blood and urine predominantly relate to homocysteine and folate, branched chain amino acid, and lipid metabolism. Statistical models are well fitted and reproducible, with excellent validation outcomes, demonstrating the feasibility of metabolic profiling to study a complex disorder with multicausal pathology, such as stroke.

Keywords

stroke, metabonomics, metabolomics, metabolic profiling, biomarker

Introduction

Stroke is a leading cause of death and disability worldwide (Donnan and others 2008). Optimizing the management of stroke patients in the hyperacute setting carries the potential to significantly improve clinical outcomes. Despite the technological advancement of modern medicine, the diagnosis of acute stroke remains a challenging entity, subject to the expertise of the attending health care provider and the diagnostic resources available (Morgenstern and others 2004). Computed tomography is only 30% sensitive in the diagnosis of acute cerebral ischemia. Diffusion-weighted magnetic resonance imaging may confer greater sensitivity; however, it is time consuming, costly, of limited availability, and may not be feasible depending on the patient’s circumstances. There remains a clinical need to develop an objective test to confer rapid and accurate diagnostic discrimination of stroke to enable the provision of rapid therapy and better direct further investigation.

Biomarkers of Stroke

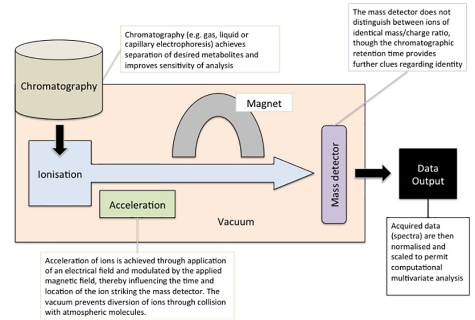
In the context of acute stroke, the ideal biomarker would permit accurate, rapid objective diagnosis of stroke, differentiate between hemorrhagic and ischemic stroke, and indicate short-term prognosis to dictate immediate management. A vast number of potential biomarkers of stroke have been investigated, but none have been incorporated into routine clinical practice (Whiteley and others 2008). Stroke is a heterogeneous condition afflicting a globally varied population and encompassing a wide array of causalities that include thrombosis, embolism, and hemorrhage. Thus, using a combination of biomarkers may be more feasible when the underlying pathology is so variable (Laskowitz and others 2009; Montaner and others 2008).

Encasing this complexity is the impermeability of the blood-brain barrier, the disruption of which in the context of stroke is nonuniform and dependent on manifold factors that include the type and magnitude of stroke. This imposes a delay in the ability of neuronal and glial proteins to reach the peripheral circulation. More generalized markers may also be released in a variety of stroke mimic conditions, thus failing in specificity (Saenger and Christenson 2010).

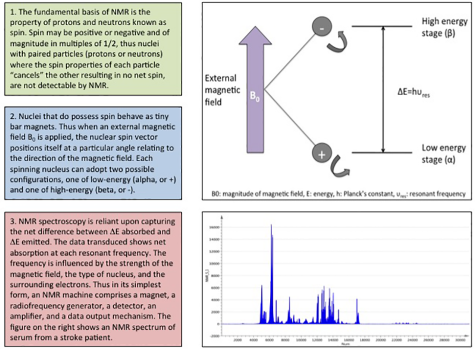
Systems Biology and Metabolic Phenotyping

Systems biology describes a holistic and integrative approach to cognizing a physiological or pathological state by assessing the “net” biological effect imparted by the incumbent condition (Shalhoub and others 2014)—whether health, disease, or intervention. It is a hierarchical science, the tiers of which represent organizational levels including DNA (genomics), RNA (transcriptomics), proteins (proteomics) through to metabolites representing endpoints of metabolism (metabonomics). Statistical relationships between gene expression and protein levels can be weak and inconsistent, and indicative only of pathophysiological potential rather than phenotype. Mechanistic elucidation of one organizational level does not confer a systematic understanding of the next level, as it does not take account of feedback mechanisms or “external” influences (e.g., drugs, environment, context) (Shalhoub and others 2014). In short, proteomics and genomics in the majority of diseases do not provide tangible endpoints for diagnostic biomarkers (Lindon and others 2003).

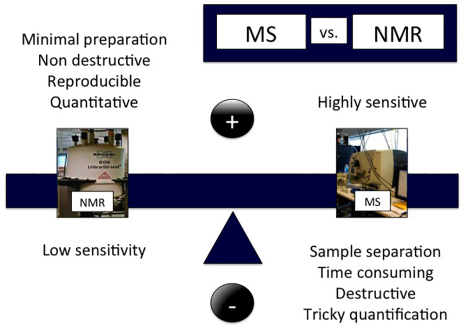
Metabonomics is defined as the quantitative measurement of the time-related multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification (Nicholson and others 1999). It is used synonymously with metabolomics or metabolic profiling and attempts to elucidate physiological or pathological pathways by rapid and comprehensive analysis of metabolic products, or metabolites, in biological specimens (Lindon and others 2003). Utilizing spectroscopic techniques—mass spectrometry (MS), usually coupled to some type of chromatographic separation (Fig. 1), and nuclear magnetic resonance (NMR) spectroscopy (Fig. 2)—metabonomics can quantify metabolites present in minute (femto- or picomolar) concentrations in biological samples. The advantages and disadvantages of each technique are summarized in Figure 3. Metabolites are small molecules (typically <1 kDa) that can cross the blood-brain barrier more readily than proteins, and they are inherently more stable than RNA or DNA. Unlike genomic, transcriptomic, or proteomic substrates that are chemically uniform, metabolites comprise a vast range of chemically distinct entities: salts, acids, bases, lipids, and so on (Issaq and others 2008). Metabonomics can within the same analysis provide a signature of biomarkers of variable nature. Coupled with the inherent link between metabolic disturbance and brain ischemic injury (Lipton 1999), metabonomics offers a distinct diagnostic advantage in the context of acute stroke diagnostics over proteomics or transcriptomics. It is a prime example of translational research where meaningful outcomes are reliant on close collaboration between clinicians and scientists (Fig. 4).



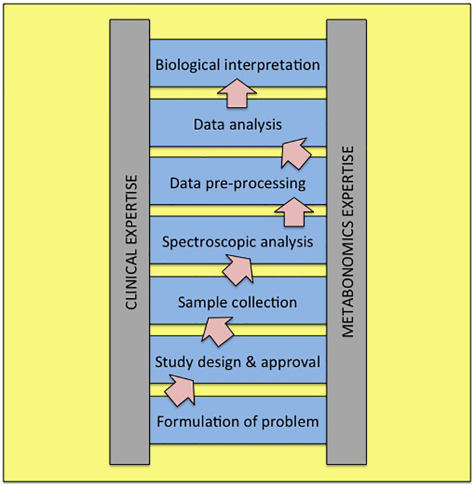
**Figure 1.** Schematic demonstrating the process of mass spectrometry in metabonomics.



**Figure 2.** Nuclear magnetic resonance spectroscopy.



**Figure 3.** The advantages and disadvantages of mass spectrometry and nuclear magnetic resonance spectroscopy.



**Figure 4.** An example of the relative input of clinicians and scientists to produce meaningful outcomes in metabonomic studies.

Metabonomics and Stroke

The application of spectroscopic techniques to study the dynamic variation of neurological metabolites is demonstrated by early studies applying high-pressure liquid chromatography coupled with MS to quantify, for example, adenosine (Melani and others 1999) and hypoxantine (Hillered and others 1991) in rat stratium extracellular fluid. Due to the limited focus of these studies and absence of a systems biology outlook, they cannot be considered metabonomics, but demonstrate the utility of spectroscopic approaches in studying metabolic molecules.

There has been a substantial increase in published studies evaluating neurobiological metabonomics in recent years. The extension of metabolic profiling to stroke research is a valid and necessary extension. Metabonomic animal studies of stroke have demonstrated biologically plausible derangement of metabolites in the context of cerebral ischemia (Gao and others 2013; Irie and others 2014; Wang and others 2014; Yang and others 2012; Zhu and others 2015). Human studies are subject to greater genetic variation (which may in turn influence metabolic profile) as well as the presence of metabolic confounders such as drugs, diet, and comorbidity. This review examines the metabolic perturbations in ischemic stroke by summarizing existing human metabonomic studies in this field.

Methods

A systematic review adhering to the principles of Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) was performed. Medline and



**Figure 5.** PRISMA diagram showing selection process for included studies.

Embase databases were searched using the Ovid Portal on May 25, 2016, with no restrictions on date of study. The search string utilized was [(metabonomics OR metabolomics OR “metabolic profiling”) AND (stroke OR “cerebrovascular attack” OR CVA OR “cerebral ischemia”)]. Two authors (MIQ and APC) performed the search independently and compared results at each stage. The senior author (AHD) arbitrated any disagreements.

Inclusion and Exclusion Criteria

All full-length English language articles utilizing MS or NMR spectroscopy for metabonomic analysis of biofluids from human stroke patients were included. The following types of studies were excluded:

1. Studies subdividing patient groups according to traditional Oriental medicine (Chinese and Korean traditional medicine)
2. Animal studies
3. Abstracts of nonpublished studies

Results

Following application of the search strategy and deduplication, 135 articles were screened, and 6 studies were included for summation analysis (Ding and others 2016; Jiang and others 2011; Jove and others 2015; Jung and others 2011; Kimberly and others 2013; Liu and others 2015) (Fig. 5). Table 1 depicts the characteristics

**Table 1.** Studies Included for Qualitative Analysis.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Author | Year | Origin | Subjects | | | | | | Biofluid | Spectroscopic Technique | Targeted/ Untargeted | Upregulated Biomarkers | Downregulated Biomarkers | Validation |
| Stroke Patients | Time from Onset to Sampling | *n* | Controls | *n* | Exclusion Criteria |
| Jung | 2011 | Korea | Cerebral infarction | ≤72 hours | 54 | Healthy volunteers | 47 | Diabetes | Plasma | H1-NMR | Untargeted | Lactate | VLDL CH3 | Training and prediction sets randomly selected and repeated three times |
|  |  |  |  |  |  |  |  | Vascular disease |  |  |  | Pyruvate | LDL CH3 |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Formate | Valine |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | Lipid CH2CH2C=C |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | Glutamine |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | Methanol |  |
|  |  |  |  |  |  |  |  |  | Urine |  |  | O-acetylcarnitine | Citrate |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Trimethylamine-NO-oxide | Dimethylamine |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Betaine | Creatine |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Carnitine | Glycine |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | Hippurate |  |
| Jiang | 2011 | China | Cerebral infarction | ≤6 hours | 67 | Routine outpatient attendees | 62 | Previous stroke | Serum | Ultra–high- pressure liquid chromatography and time of flight MS (negative ionization mode) | Untargeted | Cysteine | Folic acid | Partial least square *k*-nearest neighbor (*k* = 3); training and test division randomly carried out in 20 trials |
|  |  |  |  |  |  |  |  | Cancer |  |  |  | S-adenosyl homocysteine | Tetrahydrofolate |  |
|  |  |  |  |  |  |  |  | Cardiac insufficiency |  |  |  | Oxidized glutathione | Adenosine |  |
|  |  |  |  |  |  |  |  | Hepatosis |  |  |  | Hydroxyeicosate-traenoic acid | Aldosterone |  |
|  |  |  |  |  |  |  |  | Renal failure |  |  |  | Hydroxyocta-decadienoic acid | Deoxocathasterone |  |
|  |  |  |  |  |  |  |  | Respiratory failure |  |  |  |  | Sucrose-6-phosphate |  |
|  |  |  |  |  |  |  |  | GI hemorrhage |  |  |  |  | Betanin |  |
| Kimberly | 2013 | USA | Cardio-embolic stroke | <9 hours | 52 | TIA or nonstroke (acutely presenting patients) | 32 | None stated | Plasma | High-performance liquid chromatography | Targeted | Glucose | Leucine |  |
|  |  |  |  |  |  |  |  |  |  | HILIC MS |  |  | Isoleucine |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | Valine |  |
| Jove | 2015 | Spain | Stroke following previous TIA | ≤24 hours of  initial TIA | 35 | TIA patients that did not suffer subsequent stroke | 258 | None stated | Plasma | Liquid chromatography | Untargeted | Myristoyl-ethanolamine | 1-Monopalmitin | Second cohort of patients |
|  |  |  |  |  |  |  |  |  |  | MS |  |  | Dodecanoic acid |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | Mesoerythritol |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | Threonate |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | Lysophosphatidyl-choline (LysoPC[16:0]) |  |
| Liu | 2015 | China | Stroke or TIA | Not stated | 60 | Healthy volunteers | 20 | Prestroke cognitive impairment | Serum | Ultra–high-pressure liquid chromatography and time of flight MS |  | Carnitine | Citric acid | Separate training and  test sets |
|  |  |  |  |  |  |  |  | Hemorrhagic stroke |  |  |  | Creatine | Valine |  |
|  |  |  |  |  |  |  |  | Reduced consciousness |  |  |  | Glutamine | Isoleucine |  |
|  |  |  |  |  |  |  |  | Aphasia or dysarthria |  |  |  | Proline | Tryptophan |  |
|  |  |  |  |  |  |  |  | Acute medical illness |  |  |  | N-acetyl-neuraminic acid | LysoPCs |  |
|  |  |  |  |  |  |  |  | Cancer |  |  |  | Hypoxantine |  |  |
|  |  |  |  |  |  |  |  | Infection |  |  |  | Uric acid |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Tyrosine |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Kynureinine |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Phenylalanine |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Sphingosine-1-phosphate |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Palmitoylcarnitine |  |  |
|  |  |  | Poststroke cognitive impairment |  | 30 | Poststroke without cognitive impairment | 30 | Autoimmune disease |  |  |  | Carnitine | Valine |  |
|  |  |  |  |  |  |  |  | Acute neurological illness |  |  |  | Glutamine | Isoleucine |  |
|  |  |  |  |  |  |  |  | Psychiatric disorder |  |  |  | Uric acid | Tryptophan |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Tyrosine | LysoPCs |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Kynurenine | Palmitoylcarnitine |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Phenylalanine | Stearoylcarnitine |  |
| Ding | 2016 | China | Stroke | >14 days | 55 | Healthy volunteers | 32 | Antidepressant | Plasma | Gas chromatography | Untargeted | Aspartic acid | Palmitic acid | Separate training and  test sets |
|  |  |  |  |  |  |  |  | Mental health disorder |  | MS |  |  | Stearic acid | Univariate statistical analysis |
|  |  |  |  |  |  |  |  | Alcohol excess |  |  |  |  | Oleic acid |  |
|  |  |  |  |  |  |  |  | Metabolism disorder |  |  |  |  | Linoleic acid |  |
|  |  |  |  |  |  |  |  | Diabetes |  |  |  |  | Phenylalanine |  |
|  |  |  |  |  |  |  |  | Neurological illness |  |  |  |  | Pyroglutamate |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | Serine |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | Proline |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | Isoleucine |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | Valine |  |
|  |  |  | Poststroke depressed patients |  | 28 | Poststroke nondepressed patients | 27 |  |  |  |  | Palmitic acid | Oxalate |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Oleic acid |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Linoleic acid |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Proline |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Pyroglutamate |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  | Rhamnose |  |  |

NMR = nuclear magnetic resonance; MS = mass spectrometry; HILIC MS = hydrophilic-interaction chromatography; TIA = transient ischemic attack.

of included studies and summarizes perturbations in the metabolites of stroke patients. Three studies aimed to determine diagnostic biomarkers of stroke (Jiang and others 2011; Jung and others 2011; Kimberly and others 2013), of which one was a targeted MS study (Kimberly and others 2013), and two were untargeted MS and NMR spectroscopy studies (Jiang and others 2011; Jung and others 2011). Only one study explored biomarkers depicting recurrent stroke risk following TIA using MS (Jove and others 2015). Two studies examined the metabolic consequences of depression (Ding and others 2016) or cognitive impairment poststroke using untargeted MS (Liu and others 2015). All included studies examined blood (plasma or serum) biomarkers, and one study also analyzed urinary metabolites using NMR spectroscopy (Jove and others 2015).

Metabolic Biomarkers of Hyperacute Stroke

1H-NMR analysis of plasma and urine from 54 small vessel occlusion stroke patients compared with 47 age- and sex-matched healthy controls revealed significant differences in metabolites between the study groups (Jung and others 2011). Orthogonal Projection to Latent Structures Discriminant Analysis (OPLS-DA) revealed well-fitted models showing clear separation of stroke patients and healthy controls, with explained variability *R*2*Y* = 0.914 and predicted variability *Q*2 = 0.778 for plasma, and *R*2*Y* = 0.928, *Q*2 = 0.627, for urine. However, there were several disparities in the characteristics of the groups, as they were not matched for relevant risk factors such as previous TIA and hypertension. This study also excluded patients with concomitant vascular disease and diabetes—comorbidities present in a large proportion of stroke patients. Nonetheless, significantly elevated levels of lactate, pyruvate, and formate and decreased levels of VLDL and LDL CH3, valine, lipid CH2CH2C=C and 4-hydroxymethyl acetate were found in plasma of stroke patients. Analysis of urine revealed higher levels of O-acetylcarnitine, trimethylamine-*N*-oxide, betaine, and carnitine in stroke patients, and decreased concentrations of citrate, dimethylamine, creatine, glycine, and hippurate. External validation studies performed to test the reliability of the OPLS-DA models showed an average classification rate of 100% for stroke patients and 96% for healthy subjects (Jung and others 2011).

The reduction in circulating levels of valine in hyperacute stroke patients as well as other branched chain amino acids (leucine, isoleucine) has been demonstrated in a targeted MS analysis of plasma (Kimberly and others 2013). Fifty-two patients with mild or severe ischemic, cardioembolic stroke were compared with 32 controls (patients presenting acutely with neurological symptoms but with a final diagnosis of TIA or nonstroke). The magnitude of reduction in plasma levels of branched chain amino acids correlated with size of infarct and clinical disability in stroke patients (Kimberly and others 2013). Multivariate statistical analysis showed that branch chain amino acids were discriminatory in separating stroke from nonstroke or TIA patients, though values of *R*2*Y* and *Q*2 for PLS-DA models were not provided. Validation of the models was performed using cross-validation and permutation testing (*P* < 0.01). The significance levels of metabolites altered between stroke patients and controls were adjusted using Benjamini-Hochberg false discovery correction procedure (Kimberly and others 2013).

Increased concentrations of cysteine,   
S-adenosyl homocysteine, oxidized glutathione, hydroxyeicosaetraenoic acid, and hydroxyoctadecadienoic acid, and decreased levels of folic acid, tetrahydrofolate, adenosine, aldosterone, deoxocathasterone, sucrose-6-phosphate, and betanin were revealed in an MS analysis of serum from 67 hyperacute stroke patients and 62 controls well matched for vascular risk factors (Jiang and others 2011). The OPLS-DA model acquired was well fitted and highly predictive (*R*2*Y* = 0.998, *Q*2 = 0.947). Statistical validation was performed by applying a K-nearest neighbor (KNN) algorithm to PLS models, in which the samples were divided into a training set (to create the model) and a test set (to evaluate reliability). The location of each test sample was analyzed according to the location of its three nearest neighbors (*k* = 3), and the majority of class designations of the nearest neighbors was used to denote the identity of the test sample. This approach again revealed a prediction accuracy of 100% in discriminating stroke patients from controls.

Jove and others compared plasma metabolic profiles of TIA patients that suffered an ischemic stroke within 1 year compared with TIA patients that did not develop subsequent ischemic stroke (Jove and others 2015). Following the application of Bonferroni testing to reduce the likelihood of false positives, the discrepancies in metabolic profiles related primarily to fatty acid metabolism. Furthermore, risk of early stroke recurrence could also be differentiated from late stroke recurrence. The metabolite lysophosphocholine 20:4 increased the sensitivity of the ABCD2 score from 64% to 67% to 71% (*P* = 0.008), conferring clinical benefit (Jove and others 2015).

Two recent Chinese studies have focused on the metabonomic consequences of common neurocognitive sequelae of stroke: depression (Ding and others 2016) and cognitive impairment (Liu and others 2015). Initial comparisons of stroke patients with healthy controls echoed the findings of preceding studies in demonstrating alterations of lipid metabolism and reduction of branched chain amino acids. Significant separation was also achieved between stroke patients based on the presence or absence of depression or cognitive impairment, with well-fitted and reproducible statistical models. Liu and others (2015) identified two metabolites, 3-indolepropionic acid and stearoyl-carnitine, that were significantly reduced in stroke patients but only in the presence of cognitive impairment. However, demographic data again did not include baseline cardiovascular risk factors or pertinent medications, and particularly in the absence of false positive correction, hard conclusions cannot yet be drawn from these data.

Discussion

This review demonstrates the feasibility of metabolic profiling to further current understanding of ischemic stroke and its potential in the delivery of clinically relevant diagnostic and prognostic biomarkers, both in urine and blood. Pathways highlighted by these studies predominantly relate to homocysteine and folate, branched chain amino acid, and lipid metabolism. The disparity in discovered biomarkers between studies may reflect the diversity of applied spectroscopic techniques, range of biofluid, differences in patient populations, disparate etiologies of stroke, and variation in sampling time due to chronological variability of permeability of the blood brain barrier poststroke.

Homocysteine, a metabolite of S-adenosyl homocysteine, is a sulfur-containing amino acid synthesized from methionine (Ansari and others 2014). The regeneration of methionine from homocysteine is folate-dependent; thus, homocysteine levels are governed by genetic predisposition as well as folate and B-vitamin intake. Elevated levels of homocysteine have been associated with numerous medical disorders, including neurological disorders. As a proposed risk factor for stroke, homocysteine is hypothesized to induce oxidative injury to vascular endothelial cells, reduce the production of nitric oxide, enhance platelet adhesion to endothelial cells, and promote the growth of vascular smooth muscle cells (Jung and others 2011). Elevated homocysteine levels have been associated with poorer stroke outcomes in Chinese populations, where there is no folic acid nutritional fortification (Zhong and others 2014). However, retrospective studies have shown a stronger association between cardiovascular events and homocysteine levels than prospective studies (Homocysteine Studies Collaboration 2002). When considered with the finding that lowering homocysteine levels (e.g., with folic acid supplementation) does not appear to decrease the risk of ischemic stroke (Cacciapuoti 2013), it lends credence to the possibility of homocysteine as an acute-phase reactant.

The relevance of branched chain amino acids metabolism in relation to stroke is difficult to gauge. The patient groups in Kimberly and others’ study were representative of real-life acute attendances (making it a phase 4 study), but it focused on cardioembolic stroke (Kimberly and others 2013). Predictably patients with stroke and severe stroke were more likely to suffer from diseases such as atrial fibrillation, and it remains to be seen whether the variation in branched chain amino acids are a consequence of stroke, or a reflection of patient comorbidities, as levels of branched chain amino acids have also been shown to decrease in heart disease (Huang and others 2011).

The role of lipid metabolism is of interest in stroke research, due to the close association with atherosclerosis, the high lipid content of the central nervous system (CNS), and the ability of fatty acids to traverse the blood-brain barrier. Cerebral ischemia results in anaerobic metabolism and excess glutamate release, leading   
to activation of phospholipases/sphingomyelinases, phospholipid hydrolysis, ultimately hailing apoptopic or necrotic cell death (Adibhatla and Hatcher 2008). The association between ischemic stroke risk and fatty acid metabolism was highlighted by the prognostic stroke metabonomics study conducted by Jove and others (2015) demonstrating that markers of risk of stroke recurrence related primarily to fatty acid metabolism.

Anandamide (AEA) is a long chain fatty acid, degraded by Fatty Acid Amide Hydrolase (FAAH), and linked with neuromodulatory effects in ischemic brain injury (Esposito and others 2014). Naccarato and others used MS to compare plasma concentrations of AEA, as well as palmitoylethanolamide and 2-arachidonoyl glycerol in patients with hyperacute ischemic stroke to well-matched healthy volunteers (Naccarato and others 2010). Despite the utilization of a spectroscopic technique to determine levels of metabolites, the fact that only three compounds were assessed and without the use of multivariate statistical analysis, it cannot be considered to be metabolic profiling or metabonomics. Nevertheless, the results lend credence to the important association of lipid metabolism in CNS disease mechanism. AEA was significantly elevated in stroke patients as compared to healthy controls (3.42 ± 2.71 pmol/lipid mg vs. 1.81 ± 1.53 pmol/lipid mg; *P* < 0.05). Furthermore, plasma AEA correlated positively with size of infarct, and degree of neurological impairment (Naccarato and others 2010). AEA acts locally in the CNS on CB1 (modulating the actions of GABA and glutamate, postulated to reduce NMDA-mediated excitotoxicity in the penumbra, induce hypothermia, and reduce edema; Hillard 2008) and centrally and peripherally on CB2 receptors (immunomodulatory role triggered 24–48 hours after the onset of stroke). CB1 knock-out mice develop significantly larger stroke volumes with greater disability (England and others 2015), but significant toxicity in relation to endocannabinoids has also been reported (Pellegrini-Giampietro and others 2009); therefore, the balance of beneficial and detrimental effects of AEA requires further investigation.

Only one study examined the metabolic profile of urine, despite the relative ease of sample collection, abundant supply, and feasibility of repeat sampling. Notwithstanding these clinical benefits, the chemical composition of urine is highly variable within individuals depending on the timing of sampling. Potential urinary biomarkers of stroke are filtered twice—at the blood-brain barrier and Bowman’s capsule. The metabolic advantage is that unlike blood, urine content is not subject to homeostatic regulation, and future studies may result in new avenues of investigation yielded from urinary metabolites.

Review Limitations

This review included untargeted metabonomics studies comparing stroke patients with healthy volunteers or patients attending for routine outpatient appointments—as such they were Phase 1 and 2 diagnostic studies (Lumbreras and others 2008), and patient groups were not matched for vital characteristics, or stringent exclusion criteria imply that findings may not be translated into real-world practice. Stroke risk factors such as diabetes, hypertension, dyslipidemia, and arterial disease confer distinct metabolic profiles; it is not known whether groups matched for such characteristics would retain the significant metabolic distinctions implied by Phase 1 studies, particularly given the limited number of participants, as a small number of observations (samples) produce a large number of variables (metabolites). This results in an increased likelihood of Type I error (or a high false discovery rate), which can be statistically tempered through the application of, for example, the Benjamini-Hochberg procedure.

Furthermore, the use of pertinent medications, such as statins, was not considered by investigators: these medications will both produce signals in the plasma/serum and also induce a physiological response that may be detected in the plasma metabolome. No included study elaborated on the timing of sample collection in relation to administration of hyperacute stroke therapy (such as thrombolysis or aspirin, which might distort metabolic profiles).

The use of metabonomics as a research tool carries limitations common to diagnostic studies and systems biology studies, as well as those unique to metabolic profiling. Due to high throughput mechanisms, the cost per sample is low, but overhead maintenance and infrastructure demands are considerable (Shalhoub and others 2012). Variations in pre-analytic stages with regard to sample selection, collection, storage, and preparation can affect final results. Complex data interpretation techniques are required (Nicholson and Lindon 2008), and error can result from statistical overfitting of data (Lumbreras and others 2008), particularly with small sample sizes, although this can be mitigated by careful use of appropriate statistical validation. An appropriately adapted scoring system is required to gauge the quality of stroke metabonomics studies—prognostic as well as diagnostic.

Conclusion

Metabonomics offers a holistic overview of the physiological status of an individual and provides a feasible platform to study a complex disorder with multi-causal pathology. Despite the limited number of metabonomic stroke studies, current research spans etiological, diagnostic, and prognostic genres. Certain pathways are collectively enunciated, particularly relating to branched chain amino acid, homocysteine, folate, and lipid metabolism. In future, prospective high-quality studies with groups well matched for risk factors, stratified for stroke etiology, and with less stringent exclusion criteria are required for hyperacute biomarker delineation and improved understanding of the pathological processes that drive subtypes of stroke.

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References

Adibhatla RM, Hatcher JF. 2008. Altered lipid metabolism in brain injury and disorders. Subcell Biochem 49:241–68.

Ansari R, Mahta A, Mallack E, Luo JJ. 2014. Hyperhomocysteinemia and neurologic disorders: a review. J Clin Neurol 10(4):281–8.

Cacciapuoti F. 2013. Lowering homocysteine levels with folic acid and B-vitamins do not reduce early atherosclerosis, but could interfere with cognitive decline and Alzheimer’s disease. J Thromb Thrombolysis 36(3):258–62.

Ding X, Liu R, Li W, Ni H, Liu Y, Wu D, and others. 2016. A metabonomic investigation on the biochemical perturbation in post-stroke patients with depressive disorder (PSD). Metab Brain Dis 31(2):279–87.

Donnan GA, Fisher M, Macleod M, Davis SM. 2008. Stroke. Lancet 371(9624):1612–23.

England TJ, Hind WH, Rasid NA, O’Sullivan SE. 2015. Cannabinoids in experimental stroke: a systematic review and meta-analysis. J Cereb Blood Flow Metab 35(3):348–58.

Esposito E, Cordaro M, Cuzzocrea S. 2014. Roles of fatty acid ethanolamides (FAE) in traumatic and ischemic brain injury. Pharmacol Res 86:26–31.

Gao J, Yang H, Chen J, Fang J, Chen C, Liang R, and others. 2013. Analysis of serum metabolites for the discovery of amino acid biomarkers and the effect of galangin on cerebral ischemia. Mol Biosyst 9(9):2311–21.

Hillard CJ. 2008. Role of cannabinoids and endocannabinoids in cerebral ischemia. Curr Pharm Des 14(23):2347–61.

Hillered L, Kotwica Z, Ungerstedt U. 1991. Interstitial and cerebrospinal fluid levels of energy-related metabolites after middle cerebral artery occlusion in rats. Res Exp Med (Berl) 191(3):219–25.

Homocysteine Studies Collaboration. 2002. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. JAMA 288(16):2015–22.

Huang Y, Zhou M, Sun H, Wang Y. 2011. Branched-chain amino acid metabolism in heart disease: an epiphenomenon or a real culprit? Cardiovasc Res 90(2):220–3.

Irie M, Fujimura Y, Yamato M, Miura D, Wariishi H. 2014. Integrated MALDI-MS imaging and LC-MS techniques for visualizing spatiotemporal metabolomic dynamics in a rat stroke model. Metabolomics 10(3):473–83.

Issaq HJ, Abbott E, Veenstra TD. 2008. Utility of separation science in metabolomic studies. J Sep Sci 31(11):1936–47.

Jiang Z, Sun J, Liang Q, Cai Y, Li S, Huang Y, and others. 2011. A metabonomic approach applied to predict patients with cerebral infarction. Talanta 84(2):298–304.

Jove M, Mauri-Capdevila G, Suarez I, Cambray S, Sanahuja J, Quilez A, and others. 2015. Metabolomics predicts stroke recurrence after transient ischemic attack. Neurology 84(1):36–45.

Jung JY, Lee HS, Kang DG, Kim NS, Cha MH, Bang OS, and others. 2011. 1H-NMR-based metabolomics study of cerebral infarction. Stroke 42(5):1282–8.

Kimberly WT, Wang Y, Pham L, Furie KL, Gerszten RE. 2013. Metabolite profiling identifies a branched chain amino acid signature in acute cardioembolic stroke. Stroke 44(5):1389–95.

Laskowitz DT, Kasner SE, Saver J, Remmel KS, Jauch EC. 2009. Clinical usefulness of a biomarker-based diagnostic test for acute stroke: the Biomarker Rapid Assessment in Ischemic Injury (BRAIN) study. Stroke 40(1):77–85.

Lindon JC, Holmes E, Nicholson JK. 2003. So what’s the deal with metabonomics? Anal Chem 75(17):384A–91A.

Lipton P. 1999. Ischemic cell death in brain neurons. Physiol Rev 79(4):1431–568.

Liu M, Zhou K, Li H, Dong X, Tan G, Chai Y, and others. 2015. Potential of serum metabolites for diagnosing post-stroke cognitive impairment. Mol Biosyst 11(12):3287–96.

Lumbreras B, Porta M, Marquez S, Pollan M, Parker LA, Hernandez-Aguado I. 2008. QUADOMICS: an adaptation of the Quality Assessment of Diagnostic Accuracy Assessment (QUADAS) for the evaluation of the methodological quality of studies on the diagnostic accuracy of “-omics”-based technologies. Clin Biochem 41(16–17):1316–25.

Melani A, Pantoni L, Corsi C, Bianchi L, Monopoli A, Bertorelli R, and others. 1999. Striatal outflow of adenosine, excitatory amino acids, gamma-aminobutyric acid, and taurine in awake freely moving rats after middle cerebral artery occlusion: correlations with neurological deficit and histopathological damage. Stroke 30(11):2448–54.

Montaner J, Perea-Gainza M, Delgado P, Ribo M, Chacon P, Rosell A, and others. 2008. Etiologic diagnosis of ischemic stroke subtypes with plasma biomarkers. Stroke 39(8):2280–7.

Morgenstern LB, Lisabeth LD, Mecozzi AC, Smith MA, Longwell PJ, McFarling DA, and others. 2004. A population-based study of acute stroke and TIA diagnosis. Neurology 62(6):895–900.

Naccarato M, Pizzuti D, Petrosino S, Simonetto M, Ferigo L, Grandi FC, and others. 2010. Possible anandamide and palmitoylethanolamide involvement in human stroke. Lipids Health Dis 9:47.

Nicholson JK, Lindon JC. 2008. Systems biology: metabonomics. Nature 455(7216):1054–6.

Nicholson JK, Lindon JC, Holmes E. 1999. “Metabonomics”: understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 29(11):1181–9.

Pellegrini-Giampietro DE, Mannaioni G, Bagetta G. 2009. Post-ischemic brain damage: the endocannabinoid system in the mechanisms of neuronal death. FEBS J 276(1):2–12.

Saenger AK, Christenson RH. 2010. Stroke biomarkers: progress and challenges for diagnosis, prognosis, differentiation, and treatment. Clin Chem 56(1):21–33.

Shalhoub J, Davies KJ, Hasan N, Thapar A, Sharma P, Davies AH. 2012. The utility of collaborative biobanks for cardiovascular research. Angiology 63(5):367–77.

Shalhoub J, Sikkel MB, Davies KJ, Vorkas PA, Want EJ, Davies AH. 2014. Systems biology of human atherosclerosis. Vasc Endovascular Surg 48(1):5–17.

Wang Y, Wang YG, Ma TF, Li M, Gu SL. 2014. Dynamic metabolites profile of cerebral ischemia/reperfusion revealed by (1)H NMR-based metabolomics contributes to potential biomarkers. Int J Clin Exp Pathol 7(7):4067–75.

Whiteley W, Tseng MC, Sandercock P. 2008. Blood biomarkers in the diagnosis of ischemic stroke: a systematic review. Stroke 39(10):2902–9.

Yang M, Wang S, Hao F, Li Y, Tang H, Shi X. 2012. NMR analysis of the rat neurochemical changes induced by middle cerebral artery occlusion. Talanta 88:136–44.

Zhong C, Lv L, Liu C, Zhao L, Zhou M, Sun W, and others. 2014. High homocysteine and blood pressure related to poor outcome of acute ischemia stroke in Chinese population. PLoS One 9(9):e107498.

Zhu Y, Guo Z, Zhang L, Zhang Y, Chen Y, Nan J, and others. 2015. System-wide assembly of pathways and modules hierarchically reveal metabolic mechanism of cerebral ischemia. Sci Rep 5:17068.