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Targeted metabolomics profiles of ischemic stroke from two ethnic groups with different risk factors

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Abstract

Background— In the US, African Americans (AA) have a higher ischemic stroke and mortality rate than Caucasians. Recent evidence suggests that the onset of an ischemic stroke may be related to circulating metabolite alterations, contributing to racial disparities in ischemic stroke. The objective of this study was to determine whether the metabolomic profile of African American ischemic stroke patients differs from that of Caucasian ischemic stroke patients.

Methods— Metabolomic profiling of serum samples of 36 ischemic stroke (IS) patients (AA and Caucasian) was carried out using ultrahigh performance liquid chromatography/mass spectrometry (UHPLC/MS) to measure 1062 known metabolites. Principal component analysis (PCA) was used to differentiate global metabolite profiles for AA and Caucasian patients. A student T-test was used to compare scaled metabolite values between these two groups. One-way ANOVA identified differences in identified metabolites among the African American Ischemic stroke patients, Caucasian ischemic patients and control groups.

Results— A total of 13 metabolite levels were significantly different between the ischemic stroke and control group for African Americans, while 86 metabolite levels were significantly different between the ischemic stroke patients and control group including 36 being amino acids and 26 lipids. Of these, imidazoleacetic acid, N-acetylcysteine and alpha-hydroxyisocaproate were significantly elevated in African American and Caucasian patients as compared to controls. There was a statistically significant difference between African American and Caucasian ischemic stroke patients [(F (2,26) = 7.101, p = 0.003)] for IAA. The level was highest in the control group (1.20 ± 0.41) compared to the African American ischemic stroke patients (0.73 ± 0.38, p = 0.017) and lowest for Caucasian ischemic stroke patients (0.67 ± 0.15, p = 0.010). Alpha-hydroxyisocaproate levels were significantly different between [(F (2,26) = 7.193, p = 0.003)], and was highest for Caucasian IS patients (1.68 ± 0.89, p = 0.003), when compared with the control group (0.83 ± 0.28, p=0.05) and African American ischemic stroke patients (1.31 ± 0.3, p = 0.096). In addition, N-acetylcysteine levels were significant [(F (2,26) = 7.865, p = 0.002)], and were highest for the control group (1.30 ± 0.50, p=0.004) compared to the African American (0.69 ± 0.26, p = 0.007) and the Caucasian ischemic stroke patients (0.69 ± 0.35, p = 0.011).

Conclusions— Metabolomic profiles including imidazoleacetic acid, N-acetylcysteine and alpha-hydroxyisocaproate significantly differed between African Americans and Caucasians ischemic stroke patients. These race-associated alterations may contribute to racial disparities in the risk of ischemic stroke.

Keywords— Ischemic stroke, metabolites, metabolomics, African-Americans, Caucasian.

INTRODUCTION

Although stroke mortality has decreased over time, it remains two times higher for African Americans (AA) than White or Caucasian (non-Hispanic white) Americans[1], and African American ischemic stroke patients are more likely to present with higher severity of stroke symptoms[2-6] and show poorer outcomes compared to Caucasian patients[7-11]. These findings indicate that management of stroke risk factors is an essential,

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first-line strategy to decrease the burden of stroke in African American ischemic stroke patients.

Recent evidence reveals significant race-related differences in small-molecule metabolites detected in peripheral blood[12]. Identified differences are proposed to contribute to heterogeneity across races in various disease outcomes[12,13]. Therefore, analysis of small-molecule metabolites in African American ischemic stroke patients may reveal a possible contributing factor for the reported poor outcomes observed in this patient population[14]. Furthermore, alterations in metabolite levels may correspond to observed disparities in stroke treatment outcomes and point toward new treatment options. The analysis of biological networks and specific metabolites may also identify biomarkers to predict recovery following ischemic stroke[15].

An unbiased analysis of blood metabolites, referred to as metabolomics is a useful tool in understanding the biochemical processes that occur in the infarcted brain[16]. This analysis on routine serum samples can identify small molecule metabolites that are altered secondary to pathophysiological processes during ischemic stroke. Moreover, a metabolomics approach has been used to identify novel metabolic biomarkers for insulin resistance[17], type 2 diabetes mellitus[18], coronary artery disease[19], and incidence of cardiovascular events[19]. Recent studies have identified altered serum metabolic profiles in patients with ischemic stroke[16,20]. However, these studies did not investigate differences in metabolites between African American and Caucasian ischemic stroke patients. Therefore, no prior studies have determined differences in specific circulating small-molecule metabolites across both race-based (African Americans and Caucasian) ischemic stroke subgroups. In this study, we targeted duplicated metabolites that were expressed in the serum samples analyzed for both Caucasian and African Americans ischemic stroke patients, including; imidazole acetic acid (IAA), N-acetylcysteine (NAC), and alfa-hydroxy-isocaproic acid (HICA). Since HICA[21], NAC[24] and IAA[22,23], have been implicated in different clinical conditions, and because of differences in etiology and stroke severities in African American versus Caucasian ischemic stroke patients, we tested the hypothesis that imidazole acetic acid, N-acetylcysteine, and alfa-hydroxy-isocaproic acid would be differentially regulated in African American versus Caucasian ischemic stroke patients.

METHODS

This study consisted of ischemic stroke patients who were admitted to the Prisma Health Upstate SC, USA. Subjects that presented with ischemic stroke within 24 hours of symptom onset based on relevant ischemic lesions on computer tomography or brain MRI were included in the analysis. A total of 36 ischemic stroke patients comprising each of 18 African Americans and Caucasian ischemic stroke subjects with matched controls were recruited for this study. Serum samples from ischemic stroke patients and samples from control patients were collected. Healthy controls were randomly selected from Stroke Unit. We collected serum samples from patients within 24 hours of symptom onset based on relevant ischemic lesions on computer tomography or magnetic resonance imaging. Morning, fasting serum samples were collected in patients who are at least 18 years of age and meeting the diagnostic criteria for ischemic stroke. We excluded patients with cardiac, kidney, or liver failure, acquired immunodeficiency syndrome, inflammatory bowel disease and systemic infection. The preparation and processing of serum samples was carried out as described previously[24,25]. Briefly, individual samples were subjected to methanol extraction then split into aliquots for analysis by ultrahigh performance liquid chromatography/mass spectrometry (UHPLC/MS). Global biochemical profiling analysis was comprised of four unique arms consisting of reverse phase chromatography positive ionization methods optimized for hydrophilic compounds and hydrophobic compounds, reverse phase chromatography with negative ionization conditions as well as a HILIC chromatography method coupled to negative hydrophilic compounds.

All of the processes alternated between full-scan mass spectrometry and data-dependent MSn scans. The scan range varied slightly between methods but generally covered 70–1000 m/z. Metabolites were identified by automated comparison of the ion features in the experimental samples to a reference library of chemical standard entries that included retention time, molecular weight, preferred adducts, and in-source fragments as well as associated mass spectrometry spectra and curated by visual inspection for quality control using software developed by Metabolon Inc. Identification of known biochemical entities was based on comparison to metabolomic library entries of purified standards.

Statistical Analysis

The primary goal of our analysis was to identify metabolic differences between control and IS patients based on race and compare similarities in metabolite alterations between African American and Caucasian patients. Therefore, the two cohorts of patients (African American and Caucasians) were initially analyzed separately.

A principal component analysis (PCA) was used to visualize if the samples could be differentiated based on their global metabolite profile. Metabolites that did not have any variation between the samples were removed from this analysis. Two factors were extracted and plotted, and direct oblimin rotation was used to obtain a non-orthogonal solution for analyzing the data. After plotting samples onto a graph based on their two components, obvious outliers were removed from any further analyses based on if they visually skewed the data. A principal component analysis was performed for both African American and Caucasian ischemic stroke patients separately. Student T-tests were then used to compare the values of metabolites between ischemic stroke and control patients separately for each population. The Receiver operating curves (ROC) were developed based on the prediction that specific metabolites are from ischemic stroke samples from identified significant values. The area under the curve was used to evaluate the strength of the predictive value of each metabolite belonging to an ischemic stroke sample.

TABLE 1: Metabolite differences between ischemic stroke patients and control patients in the African American population. Levels were standardized before the analysis with a median of 1.

Metabolites	Super Pathway	Sub Pathway	Ischemic	Control	P-value
1-ribosyl-imidazoleacetate	Amino Acid	Histidine Metabolism	0.91 ± 0.72	2.88 ± 2.36	0.046
Alpha-hydroxyisocaproate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	1.31 ± 0.3	0.89 ± 0.28	0.014
Isovalerate (C5)	Amino Acid	Leucine, Isoleucine and Valine Metabolism	0.62 ± 0.3	1.2 ± 0.45	0.027
N-acetylhistidine	Amino Acid	Histidine Metabolism	0.76 ± 0.3	1.61 ± 0.78	0.022
N-carbamoylalanine	Amino Acid	Alanine and Aspartate Metabolism	0.69 ± 0.26	1.28 ± 0.58	0.01
Tryptophan betaine	Amino Acid	Tryptophan Metabolism	0.73 ± 0.38	1.14 ± 0.33	0.042
Glucuronate	Carbohydrate	Aminosugar Metabolism	0.73 ± 0.24	1.24 ± 0.34	0.005
1-oleoyl-2-docosahexaenoyl-GPE (18:1/22:6)	Lipid	Phosphatidylethanolamine (PE)	1.72 ± 0.73	0.92 ± 0.25	0.039
Arachidonoylcholine	Lipid	Fatty Acid Metabolism (Acyl Choline)	0.81 ± 1	3.05 ± 2.72	0.041
Cis-3,4-methyleneheptanoate	Lipid	Fatty Acid, Branched	0.74 ± 0.79	2.51 ± 2.06	0.017
Cortisone	Lipid	Corticosteroids	1.43 ± 0.76	0.73 ± 0.29	0.043
Oleoylcholine	Lipid	Fatty Acid Metabolism (Acyl Choline)	1.32 ± 0.51	0.77 ± 0.43	0.092
1,3,7-trimethylurate	Xenobiotics	Xanthine Metabolism	0.63 ± 0.39	0.26 ± 0	0.035

TABLE 2: Metabolite differences between ischemic stroke patients and control patients in the Caucasian population.

Metabolites	Super Pathway	Sub Pathway	Ischemic	Control	P-value
N6-acetyllysine	Amino Acid	Lysine Metabolism	0.82 ± 0.31	1.27 ± 0.32	0.02
N-acetylserine	Amino Acid	Glycine, Serine and Threonine Metabolism	0.73 ± 0.2	1.31 ± 0.3	0.001
Hydroxy-N6,N6,N6-trimethyllysine	Amino Acid	Lysine Metabolism	0.76 ± 0.19	1.31 ± 0.41	0.011
5-hydroxylysine	Amino Acid	Lysine Metabolism	0.85 ± 0.39	1.84 ± 0.94	0.033
5-(galactosylhydroxy)-L-lysine	Amino Acid	Lysine Metabolism	0.85 ± 0.27	1.76 ± 0.49	0.001
N-acetylphenylalanine	Amino Acid	Phenylalanine Metabolism	0.72 ± 0.14	2.05 ± 1.41	0.046
1-carboxyethylphenylalanine	Amino Acid	Phenylalanine Metabolism	0.9 ± 0.68	2.31 ± 1.45	0.038
4-hydroxyphenylpyruvate	Amino Acid	Tyrosine Metabolism	0.79 ± 0.3	1.3 ± 0.34	0.011
Vanillactate	Amino Acid	Tyrosine Metabolism	0.4 ± 0.1	0.76 ± 0.35	0.036
Vanillylmandelate (VMA)	Amino Acid	Tyrosine Metabolism	0.7 ± 0.31	1.36 ± 0.56	0.018
C-glycosyltryptophan	Amino Acid	Tryptophan Metabolism	0.86 ± 0.17	1.37 ± 0.38	0.006
5-hydroxyindoleacetate	Amino Acid	Tryptophan Metabolism	0.42 ± 0.18	1.04 ± 0.48	0.007
N-acetylalanine	Amino Acid	Alanine and Aspartate Metabolism	0.95 ± 0.11	1.25 ± 0.21	0.006
Alpha-hydroxyisocaproate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	1.68 ± 0.89	0.78 ± 0.3	0.038
N-acetylvaline	Amino Acid	Leucine, Isoleucine and Valine Metabolism	0.87 ± 0.18	1.09 ± 0.2	0.048
2,3-dihydroxy-2-methylbutyrate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	0.89 ± 0.42	1.77 ± 0.5	0.004
Hydroxyasparagine	Amino Acid	Alanine and Aspartate Metabolism	0.84 ± 0.14	1.49 ± 0.36	0.001
N-formylmethionine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	0.85 ± 0.15	1.37 ± 0.26	0.001

TABLE 2: Continued.

Metabolites	Super Pathway	Sub Pathway	Ischemic	Control	P-value
S-adenosylhomocysteine (SAH)	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	0.77 ± 0.21	1.27 ± 0.49	0.03
2,3-dihydroxy-5-methylthio-4-pentenoate (DMTPA)	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	0.78 ± 0.19	1.55 ± 0.72	0.03
Cystathionine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	0.77 ± 0.33	1.46 ± 0.71	0.044
Cysteine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	1.02 ± 0.2	1.73 ± 0.54	0.012
3-sulfo-L-alanine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	0.77 ± 0.33	1.47 ± 0.47	0.008
Proline	Amino Acid	Urea cycle; Arginine and Proline Metabolism	1.01 ± 0.22	1.31 ± 0.21	0.027
Dimethylarginine (ADMA + SDMA)	Amino Acid	Urea cycle; Arginine and Proline Metabolism	0.96 ± 0.11	1.13 ± 0.15	0.03
N-acetylarginine	Amino Acid	Urea cycle; Arginine and Proline Metabolism	0.87 ± 0.37	1.45 ± 0.49	0.026
4-hydroxyglutamate	Amino Acid	Glutamate Metabolism	0.81 ± 0.57	1.98 ± 0.94	0.015
Creatinine	Amino Acid	Creatine Metabolism	0.97 ± 0.13	1.23 ± 0.26	0.037
5-methylthioadenosine (MTA)	Amino Acid	Polyamine Metabolism	0.68 ± 0.17	1.75 ± 0.88	0.008
Beta-citrylglutamate	Amino Acid	Glutamate Metabolism	0.67 ± 0.29	1.12 ± 0.39	0.029
4-acetamidobutanoate	Amino Acid	Polyamine Metabolism	0.79 ± 0.24	1.22 ± 0.33	0.015
Cysteine-glutathione disulfide	Amino Acid	Glutathione Metabolism	0.74 ± 0.36	1.15 ± 0.33	0.047
2-aminobutyrate	Amino Acid	Glutathione Metabolism	1.81 ± 0.83	0.99 ± 0.28	0.029
N-acetylhistidine	Amino Acid	Histidine Metabolism	0.69 ± 0.35	1.32 ± 0.45	0.013
1-methyl-4-imidazoleacetate	Amino Acid	Histidine Metabolism	0.89 ± 0.25	1.29 ± 0.37	0.036
1-ribosyl-imidazoleacetate	Amino Acid	Histidine Metabolism	0.67 ± 0.15	1.26 ± 0.49	0.01
Maltose	Carbohydrate	Glycogen Metabolism	0.74 ± 0.22	1.3 ± 0.37	0.005
Erythronate	Carbohydrate	Aminosugar Metabolism	0.86 ± 0.21	1.57 ± 0.68	0.021
Gamma-CEHC glucuronide	Cofactors and Vitamins	Tocopherol Metabolism	0.46 ± 0.03	1.4 ± 0.91	0.035
Succinylcarnitine (C4-DC)	Energy	TCA Cycle	0.67 ± 0.27	1.2 ± 0.38	0.011
Arachidate (20:0)	Lipid	Long Chain Saturated Fatty Acid	1.07 ± 0.13	1.26 ± 0.17	0.037
2-aminoheptanoate	Lipid	Fatty Acid, Amino	0.6 ± 0.15	1.27 ± 0.3	<0.001
2-methylmalonylcarnitine (C4-DC)	Lipid	Fatty Acid Metabolism (also BCAA Metabolism)	0.64 ± 0.25	1.15 ± 0.48	0.028
Octadecanedioylcarnitine (C18-DC)	Lipid	Fatty Acid Metabolism (Acyl Carnitine, Dicarboxylate)	1.32 ± 0.55	0.71 ± 0.34	0.028
Deoxycarnitine	Lipid	Carnitine Metabolism	0.87 ± 0.22	1.19 ± 0.22	0.017
N-oleoyltaurine	Lipid	Endocannabinoid	1.45 ± 0.87	0.48 ± 0.32	0.026
1-linoleoyl-2-linolenoyl-GPC (18:2/18:3)	Lipid	Phosphatidylcholine (PC)	0.91 ± 0.74	2.28 ± 1.43	0.043
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	Lipid	Phosphatidylethanolamine (PE)	0.94 ± 0.43	1.93 ± 0.96	0.029
1-oleoyl-2-linoleoyl-GPE (18:1/18:2)	Lipid	Phosphatidylethanolamine (PE)	0.77 ± 0.51	2.53 ± 1.44	0.017
1,2-dilinoleoyl-GPE (18:2/18:2)	Lipid	Phosphatidylethanolamine (PE)	0.63 ± 0.72	3.42 ± 2.35	0.02
1-palmitoyl-GPA (16:0)	Lipid	Lysophospholipid	0.93 ± 0.28	1.41 ± 0.46	0.039

TABLE 2: Continued.

Metabolites	Super Pathway	Sub Pathway	Ischemic	Control	P-value
1-linoleoyl-GPA (18:2)	Lipid	Lysophospholipid	0.84 ± 0.45	2.08 ± 1.17	0.032
1-linoleoyl-GPE (18:2)	Lipid	Lysophospholipid	1.1 ± 0.76	2.2 ± 0.95	0.033
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPC (P-16:0/20:4)	Lipid	Plasmalogen	1.11 ± 0.27	0.82 ± 0.17	0.029
1-linoleoylglycerol (18:2)	Lipid	Monoacylglycerol	1.15 ± 0.59	1.94 ± 0.64	0.033
1-linolenoylglycerol (18:3)	Lipid	Monoacylglycerol	1.07 ± 0.62	2.01 ± 0.84	0.035
Glycosyl-N-(2-hydroxynervonoyl)-sphingosine (d18:1/24:1(2OH))	Lipid	Hexosylceramides (HCER)	0.83 ± 0.38	1.36 ± 0.46	0.034
Glycosyl-N-tricosanoyl-sphingadienine (d18:2/23:0)	Lipid	Hexosylceramides (HCER)	0.9 ± 0.65	1.78 ± 0.59	0.021
N1-methylinosine	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	0.6 ± 0.31	1.68 ± 0.79	0.01
Allantoin	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	0.76 ± 0.44	1.27 ± 0.39	0.041
N6-carbamoylthreonyladenosine	Nucleotide	Purine Metabolism, Adenine containing	0.89 ± 0.09	1.44 ± 0.46	0.019
N6-succinyladenosine	Nucleotide	Purine Metabolism, Adenine containing	0.8 ± 0.21	1.39 ± 0.49	0.013
7-methylguanine	Nucleotide	Purine Metabolism, Guanine containing	0.94 ± 0.15	1.18 ± 0.21	0.034
N2,N2-dimethylguanosine	Nucleotide	Purine Metabolism, Guanine containing	0.86 ± 0.14	1.47 ± 0.59	0.034
Orotidine	Nucleotide	Pyrimidine Metabolism, Orotate containing	0.78 ± 0.19	1.64 ± 0.75	0.022
Uridine	Nucleotide	Pyrimidine Metabolism, Uracil containing	1.23 ± 0.2	0.97 ± 0.17	0.024
Pseudouridine	Nucleotide	Pyrimidine Metabolism, Uracil containing	0.88 ± 0.11	1.38 ± 0.38	0.013
5,6-dihydrouridine	Nucleotide	Pyrimidine Metabolism, Uracil containing	0.88 ± 0.18	1.48 ± 0.44	0.006
3-(3-amino-3-carboxypropyl)uridine	Nucleotide	Pyrimidine Metabolism, Uracil containing	0.87 ± 0.16	1.42 ± 0.53	0.034
Cytidine	Nucleotide	Pyrimidine Metabolism, Cytidine containing	0.72 ± 0.26	1.4 ± 0.6	0.017
N4-acetylcytidine	Nucleotide	Pyrimidine Metabolism, Cytidine containing	0.87 ± 0.26	1.37 ± 0.46	0.027
4-hydroxyphenylacetylglutamine	Peptide	Acetylated Peptides	0.19 ± 0.11	1.08 ± 0.83	0.029
Gamma-glutamylalanine	Peptide	Gamma-glutamyl Amino Acid	0.76 ± 0.59	1.54 ± 0.72	0.049
Gamma-glutamylglycine	Peptide	Gamma-glutamyl Amino Acid	0.8 ± 0.57	1.61 ± 0.71	0.035
Glycine conjugate of C10H12O2	Partially Characterized Molecules	Partially Characterized Molecules	0.52 ± 0.21	1.1 ± 0.53	0.02
Pentose acid	Partially Characterized Molecules	Partially Characterized Molecules	0.69 ± 0.43	2.58 ± 1.79	0.031
Gamma-glutamylphenylalanine	Peptide	Gamma-glutamyl Amino Acid	0.89 ± 0.37	1.33 ± 0.38	0.048
Gamma-glutamylcitrulline	Peptide	Gamma-glutamyl Amino Acid	0.76 ± 0.44	1.79 ± 0.6	0.003
Prolylglycine	Peptide	Dipeptide	0.73 ± 0.6	2.11 ± 1.27	0.03
4-hydroxyhippurate	Xenobiotics	Benzoate Metabolism	0.58 ± 0.3	1.81 ± 1.15	0.03
4-allylcatechol sulfate	Xenobiotics	Benzoate Metabolism	0.26 ± 0	1.22 ± 0.88	0.028
O-cresol sulfate	Xenobiotics	Benzoate Metabolism	0.92 ± 0.31	1.51 ± 0.59	0.037

TABLE 3: Post Hoc Analysis of 3 serum metabolites with ischemic stroke in the Circulating Biomarkers in Blacks, Caucasian Acute Ischemic stroke patients.

Dependent Variable	Race	Race	Mean Difference	Std. Error	Sig.
1-ribosyl-imidazoleacetate	Ischemic_AA	Ischemic_C	0.06	0.18	0.945
		Control	-0.47	0.16	0.017*
	Ischemic_C	Ischemic_AA	-0.06	0.18	0.945
		Control	-0.53	0.16	0.01*
	Control	Ischemic_AA	0.47	0.16	0.017*
		Ischemic_C	0.53	0.16	0.01*
Alpha-hydroxyisocaproate	Ischemic_AA	Ischemic_C	-0.37	0.26	0.34
		Control	0.48	0.22	0.096
	Ischemic_C	Ischemic_AA	0.37	0.26	0.34
		Control	0.85	0.23	0.003*
	Control	Ischemic_AA	-0.48	0.22	0.096
		Ischemic_C	-0.85	0.23	0.003*
N-acetylhistidine	Ischemic_AA	Ischemic_C	-0.01	0.21	>0.999
		Control	-0.61	0.18	0.007*
	Ischemic_C	Ischemic_AA	0.01	0.21	>0.999
		Control	-0.6	0.19	0.011*
	Control	Ischemic_AA	0.61	0.18	0.007*
		Ischemic_C	0.6	0.19	0.011*

Abbreviations: C= Caucasians, AA=African-Americans.

FIGURE 1: Principal Component Analysis for all metabolites in the African American ischemic and control population. Component 1 is plotted on the X axis and Component 2 is plotted on the Y axis. Component 1 encompassed 24.00% of the variation between participants, and Component 2 encompassed 11.93% of the variation between participants.

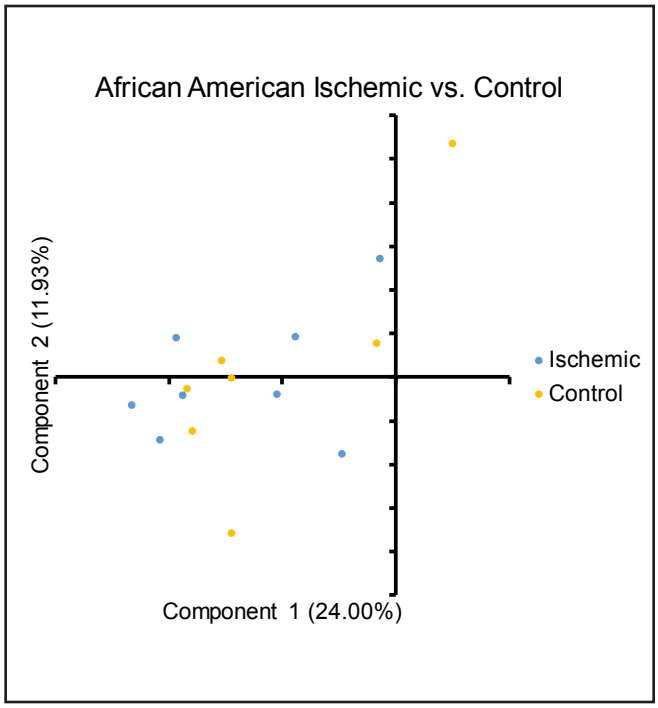
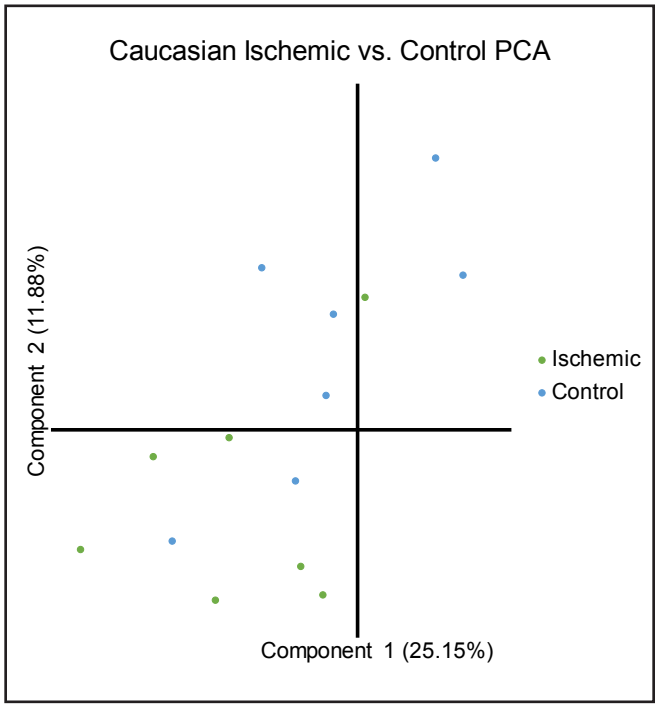


FIGURE 2: Principal Component Analysis for all metabolites in the Caucasian ischemic and control population. Component 1 is plotted on the X axis, and Component 2 is plotted on the Y axis. Component 1 encompassed 25.15% of the variation between participants, and Component 2 encompassed 11.88% of the variation between participants..



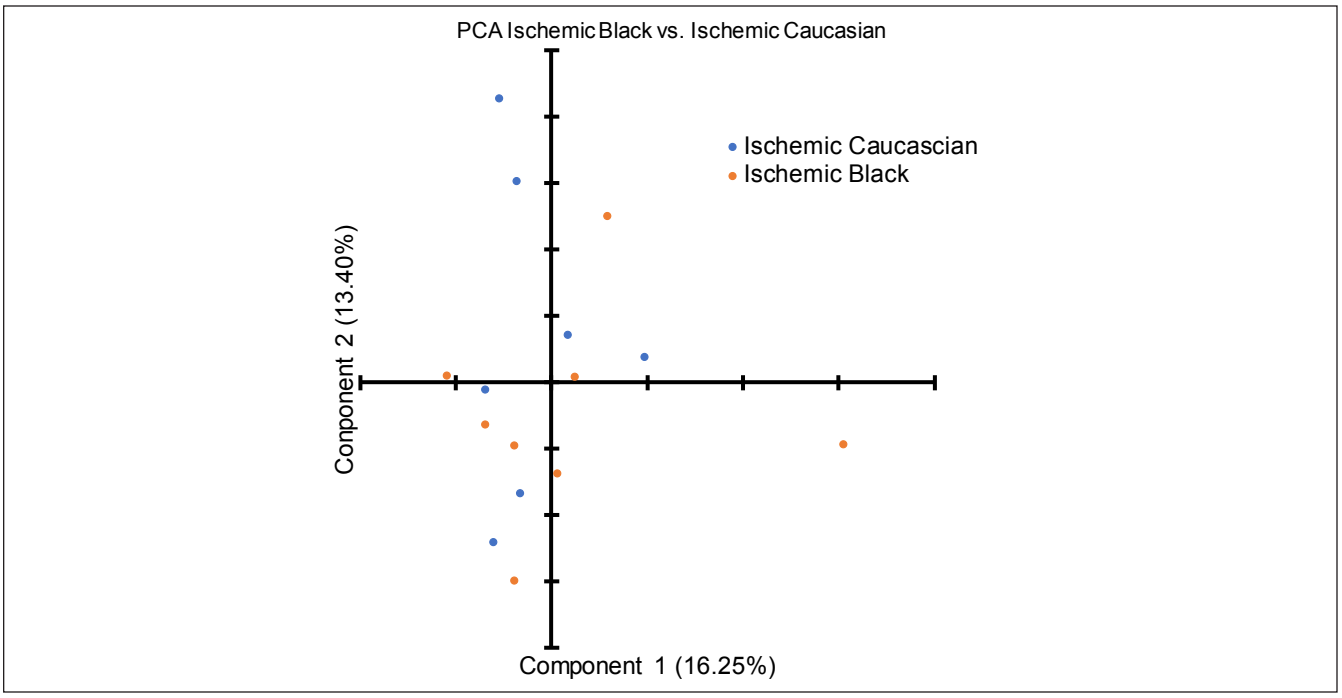


FIGURE 3: Principal Component Analysis for all metabolites in the Caucasian ischemic and African American population. Component 1 is plotted on the X axis and Component 2 is plotted on the Y axis. Component 1 encompassed 16.25% of the variation between participants and Component 2 encompassed 13.40% of the variation between participants.

Another PCA was computed for ischemic stroke African Americans and Caucasian groups similarly as described above. The same outliers were not included in this analysis. From the preliminary analysis of the metabolite levels differences, metabolites that were significantly different from the control group for the African American population were compared to the Caucasian patient population. Metabolites that had significant detection in both groups, including; imidazole acetic acid, N-acetylcysteine, and alfa-hydroxy-isocaproic acid were then targeted for further comparison between African American and Caucasian patients. A one-way ANOVA was used to determine differences between African American ischemic stroke, Caucasian ischemic stroke, and control groups. Post Hoc analysis using the Tukey method was performed to determine significant differences between the three groups. The Receiver operating curves were plotted for the metabolites with either ischemic stroke or control based on if the ischemic samples were positive or negative. The area under the curve was used as a quantitative marker of the strength of the predictive value for each metabolite. All analyses were performed using SPSS Version 26.0, and all variables were screened for outliers and univariate normality.

RESULTS

The present dataset comprised of 1322 metabolites, 1062 compounds of known identity (named metabolites), and 260 compounds of unknown structural identity (unnamed metabolites). A total of 31 patients were included in this analysis. In the African American population, 7 were controls, and 9 were ischemic stroke patients. In the Caucasian population, 8 were controls, and 7 were ischemic stroke patients. For the African American population (Fig 1), the PCA did not reveal separation between the ischemic and control groups. It is possible that it is because of the control selection, that was not originally designed for metabolomics study. This may introduce selection bias, especially if not properly accounted for and could induce the bias in the metabolite-phenotype relationships in selected groups and affect the results.

As shown in Fig 1, component 1 encompassed 24.00% of the variance while component 2 encompassed 11.93% of the data. A total of 13 metabolites were significantly different between the ischemic stroke patients and control group (Table 1). Specifically, alpha-hydroxyisocaproate, 1-oleoyl-2-docosahexaenoyl-GPE (18:1/22:6), cortisone, oleoylcholine, and 1,3,7-trimethylurate were all elevated in the ischemic stroke patient samples compared to the control samples. However, 1-ribosyl-imidazoleacetate, isovalerate (C5), N-acetylhistidine, N-carbamoylalanine, tryptophan betaine, glucuronate, arachidonoylcholine, and cis-3,4-methyleneheptanoate were all lower in the IS group compared to the control group. Alpha-hydroxyisocaproate (AUC = 0.857, 0.643-1.000), cis-3,4-methyleneheptanoate Area under the curve (AUC = 0.929, 0.782-1.000), and cortisone (AUC = 0.839, 0.630-1.000) were all predicted to be strongly associated with IS.

For the Caucasian population, the PCA did not reveal separation between the ischemic and control groups based on overarching components. A control sample was removed from the remainder of the analyses due to being an outlier on PCA. Component 1 encompassed 25.15% of the variance, and component 2 encompassed 11.88% of the data (Fig 2). Eighty -six metabolites were significantly different between the ischemic and control groups, including amino acid group, lipids, and nucleotide metabolites (Table 2). The table summarizes the different metabolites and highlighted differences between ischemic stroke patients and

control patients in the Caucasian population The predictive value for 2-aminobutyrate (AUC = 0.878, 0.650-1.000), N-oleoyltaurine (AUC = 0.816, 0.569-1.000), and uridine (AUC = 0.857, 0.651-1.000) were all strongly associated with ischemic stroke. The PCA did not reveal separation between the African American and Caucasian groups based on overarching components (Fig 3). As shown in the figure, component 1 encompassed 16.25% of the variance whereas, component 2 encompassed 13.40% of the data.

The three duplicated metabolites in African American and Caucasian patients including imidazole acetic acid, N-acetylcysteine, and alfa-hydroxy-isocaproic acid were subjected to variance analysis. There was statistically significant difference between African American and Caucasian ischemic stroke patients [(F (2,26) = 7.101, p = 0.003)] for the imidazoleacetic acid metabolite. A Tukey post hoc test revealed that the control group metabolite level (1.20 ± 0.41) was significantly higher when compared to the African American (0.73 ± 0.38, p = 0.017) and the Caucasian ischemic stroke groups (0.67 ± 0.15, p = 0.010). There also was a statistically significant difference between African American and Caucasian patient populations [(F (2,26) = 7.193, p = 0.003)] for alpha-hydroxyisocaproate. A Tukey post hoc test revealed that the control group alpha-hydroxyisocaproate metabolite level (0.83 ± 0.28, p=0.05) was statistically significantly lower compared to the Caucasians ischemic stroke patients (1.68 ± 0.89, p = 0.003), but not to the African Americans (1.31 ± 0.3, p = 0.096). Moreover, there also was a significant difference between African American and Caucasian ischemic stroke patients [(F (2,26) = 7.865, p = 0.002)] for N-acetylcysteine. A Tukey post hoc test revealed that the control group N-acetylcysteine metabolite level (1.30 ± 0.50, p=0.004) was significantly higher compared to the African American (0.69 ± 0.26, p = 0.007) and the Caucasian ischemic stroke groups (0.69 ± 0.35, p = 0.011). For the imidazoleacetic acid metabolite, area under the ROC curve for the African American group (AUC = 0.830, 0.630-1.031) was significant (P = 0.001), and for Caucasian ischemic stroke patients, the area under the ROC curve (AUC = 0.949, 0.861-1.037) was significant (P <0.001). In addition, for the alpha-hydroxyisocaproate metabolite, the area under the ROC curve for African American ischemic stroke patients (AUC = 0.893, 0.759-1.027) was significant (P <0.001) and the area under the ROC curve for Caucasian ischemic stroke patients (AUC = 0.765, 0.506-1.024) was significant (P = 0.045). Lastly, for African American ischemic stroke patients, the area under the curve (AUC = 0.875, 0.730-1.020) was significant (P <0.001), and for Caucasian patients, the area under the ROC curve (AUC = 0.847, 0.667-1.027) was significant (P <0.001) for the N-acetylcysteine metabolite level.

DISCUSSION

Metabolomics data analysis is an effective strategy that can detect changes in small-molecule metabolites in a large number of metabolic pathways[26]. Since it is challenging to detect metabolites directly in the brain, metabolite analysis in serum is an effective and alternative indicator to reflect biological and pathological activities in the brain[27]. Therefore, metabolic changes in the brain can alter the metabolome of biofluids[28]. In particular, metabolites with low molecular weight can quickly diffuse from the cerebrospinal fluid and the blood[29]. Therefore, potential biomarkers associated with stroke onset and recovery can be identified in the serum using a metabolomics approach. In addition, this analytical method for stroke patient serum metabolites allows the metabolic pathways that correspond with the pathological changes associated with ischemic stroke to be identified. Most of the existing studies of metabolomic biomarkers in patients with ischemic stroke currently compare metabolomic profiles of patients within the control population[26] and among ischemic stroke patients with excellent or poor functional recovery[30] or those undergoing acute ischemic stroke inpatient rehabilitation[31]. The current study identified imidazole acetic acid, N-acetylcysteine, and alfa-hydroxy-isocaproic acid as metabolites expressed in Caucasian and African American ischemic stroke patients.

We observed that levels of imidazoleacetic acid were higher than in the control group compared to the ischemic stroke patients and were not significantly different between African American and Caucasian patients. Imidazole-4-acetic acid is a γ-aminobutyric acid type A receptor agonist[32]. In the central nervous system, imidazoleacetic acid mediates other effects, including analgesia, sedation, hypnosis, aggression, and hypotension[33]. Imidazole-4-acetic acid is also present in the brain, where its levels increase after the inhibition of histamine methyltransferase[32]. The brain oxidizes histamine and form imidazole-4-acetic acid; therefore, the formation of imidazole-4-acetic acid, a potent GABA-A agonist with numerous neurochemical effects, can affect the physiological functions in the central nervous system. While the levels of imidazoleacetic acid are known to be highly upregulated in Alzheimer disease[23], levels of imidazole-4-acetic acid were significantly lower in African American, and Caucasian ischemic stroke patients compared with the control group. This finding suggests that while a role for imidazole-4-acetic acid has been linked to Parkinson's disease[34] and Alzheimer's disease[23], its effect might not be directly associated with pathological changes in ischemic stroke patients. Therefore, an experimental study is necessary to determine if levels of imidazole-4-acetic acid have a physiological role impacting ischemic stroke patients.

We observed that the alpha-hydroxyisocaproate metabolite was significantly upregulated in the Caucasian ischemic stroke group when compared to the African American ischemic stroke group and lowest in the control group. Alpha-hydroxyisocaproate is an end product of leucine metabolism in human tissues such as muscle and connective tissue[35]. Alpha-hydroxyisocaproate effectively relieves the symptoms of delayed onset muscle soreness and protects muscle from catabolism[36]. Therefore, alpha-hydroxyisocaproate functions as an "anti-catabolite" and has shown some potential as a topical antibiotic[37]. Upregulated levels of alpha-hydroxyisocaproate have been found in the urine of patients with dihydrolipoyl dehydrogenase (E3) deficiency[38]. Alpha-hydroxyisocaproate is also elevated in maple syrup urine disease and has been shown to accelerate lipid peroxidation. In addition, alpha-

hydroxyisocaproate is an indicator of oxidative stress in several clinical conditions[21], as it attenuates the chronic inflammatory response[39]. Alpha-hydroxyisocaproate facilitates protein synthesis to improve muscle recovery following an injury[22] and could be implicated in the upstream or downstream ischemic stroke pathology. Our finding of upregulated alpha-hydroxyisocaproate in Caucasian ischemic stroke patients, when compared to African American ischemic stroke patients, suggests that Alpha-hydroxyisocaproate may be linked with ischemic stroke among Caucasian patients. Even in skeletal muscle where alpha-hydroxyisocaproate levels are high, the benefits of alpha-hydroxyisocaproate are not fully known[40]. A relatively low basal protein synthesis caused by pretreatment with alpha-hydroxyisocaproate led to the suppression of acute inflammatory responses such as iNOS and IL-6 overexpression, and ubiquitin-proteasome system's downregulation was reported[40]. Therefore, alpha-hydroxyisocaproate is proposed to improve systemic inflammation because AMPK activation generally suppresses inflammation in several tissues[41]. The suppression of systemic inflammation leads to the maintenance of the muscle mass via increased energy efficiency, and it decreases the exposure of skeletal muscle to proinflammatory cytokines[42]. It is also possible that systemic effects of alpha-hydroxyisocaproate may lead to facilitating the suppression of systemic inflammation among Caucasian ischemic stroke patients compared with African American ischemic stroke patients. A future study on alpha-hydroxyisocaproate among African American ischemic stroke patients could help elucidate how the health benefits of alpha-hydroxyisocaproate potentially through introduction of fermented foods could contribute to the extension of a healthy life in African American ischemic stroke patients.

We observed that N-acetylcysteine levels in the control group were significantly upregulated compared with the African American and Caucasian ischemic stroke patients, which were not significantly different. Oxidative stress is part of ischemic stroke's pathogenic mechanism, and N-acetylcysteine exhibits both direct and indirect antioxidant properties[43]. The direct effect of N-acetylcysteine is associated with the free thiol group that interacts with and scavenges reactive oxygen species[44], while its indirect antioxidant effect is linked to its role as glutathione precursor.

A high dose of N-acetylcysteine is reported to alleviate oxidative stress and inflammatory response in chronic obstructive pulmonary disease patients[45]. In addition, treatment with N-acetylcysteine significantly prevented TNF-a production in alveolar macrophages treated with ultrafine nickel particles[46]. Moreover, high-dose N-acetylcysteine therapy is beneficial to H1N1 influenza pneumonia patients[47]. Oxidative stress contributes to the pathogenic mechanism of ischemic stroke. N-acetylcysteine is associated with oxidative stress and the inflammatory response[24] and may contribute to the different etiologies and ischemic stroke severity. Our finding that N-acetylcysteine is upregulated in Caucasian and African American ischemic stroke patients suggests that lowering oxidative stress in ischemic stroke patients maybe be associated with treatment with N-acetylcysteine to mitigate oxidative stress, inflammatory factors. This possibility is supported by other studies that demonstrated N-acetylcysteine's potential antioxidant and anti-inflammatory properties in several clinical conditions, including chronic obstructive pulmonary disease[48] and Pneumonia[49]. A high dose of N-acetylcysteine was reported to improve clinical outcomes of obstructive pulmonary disease exacerbation patients by ameliorating oxidative stress and inflammatory responses, thereby improving oxygenation[50]. Since N-acetylcysteine has the ability to regulate inflammatory actions, such that IL-8, IL-6, and TNF l[51], future studies are necessary to determine whether treatment with N-acetylcysteine might reduce oxidative and inflammatory damage in ischemic stroke patients.

In the current study, we determined metabolomic differences in African Americans versus Caucasian ischemic stroke patients. We also targeted duplicated metabolites that were expressed in the serum samples analyzed for both Caucasian and African American ischemic stroke patients, including; imidazole acetic acid, N-acetylcysteine and alfa-hydroxy-isocaproic acid. Imidazole acetic acid is known to be present in the brain and cerebrospinal fluid, and its levels in the brain increase after inhibition of the histamine-methyltransferase enzyme. The brain is known to have the capacity to oxidize histamine and generate imidazoleacetic acid[52]. Imidazole acetic is a potent GABA-A agonist with numerous neurochemical effects, including physiological and pathophysiological functions, and is robustly expressed in the CNS and the periphery[53]. Imidazole acetic levels have been found to be highly upregulated in patients with Alzheimer's disease compared with controls[22,23], suggesting imidazole acetic's role in different neurological conditions. Because of differences in etiology and stroke severities in African Americans versus Caucasian ischemic stroke patients, we tested the hypothesis that imidazole acetic acid, N-acetylcysteine and alfa-hydroxy-isocaproic acid would be differentially regulated in African American versus Caucasian IS patients. We observed significant differences in the upregulation of levels of imidazole acetic acid, N-acetylcysteine and alfa-hydroxy-isocaproic acid in ischemic stroke patients with two different ethnic groups including African American and Caucasian ischemic stroke patients. Identifying the unique metabolic characteristics of ischemic stroke in African Americans and Caucasian patients may highlight the biological relevance and implications of investigating mechanistic differences across other ethnic groups, which could be used to improve stroke outcomes for all patients.

LIMITATIONS

Patients for this study were from a single, urban stroke unit, therefore, our results cannot be generalized to the general population. Although, the stratification approach used in this study improved the ability to detect extremes values; nevertheless, relatively small sample size is a limitation to this study. Differences in metabolites between African American and Caucasian ischemic stroke patients could be due to several other metabolites or other factors. In the current study, we identified metabolites that had significant

detection in both groups, including; imidazole acetic acid, N-acetylcysteine, and alfa-hydroxy-isocaproic acid. We targeted these metabolites for further comparison between African American and Caucasian ischemic stroke patients and observed major differences in their levels of expression. The biological mechanisms underlying the observed differences should be the subject of further studies.

CONCLUSION

The current study identified the different metabolites and metabolic pathways in African Americans and Caucasian ischemic stroke patients using the metabolomics approach. We demonstrated that metabolomics might be used to distinguish metabolites in two ethnic groups with differing risk factors for ischemic stroke. Our findings highlight the potential of metabolomics to reveal new pathways for ischemic stroke and provide a new avenue to explore ischemic stroke's pathophysiological mechanisms in different ethnic groups with various risk factors for ischemic stroke. Moreover, identification of specific metabolites in our ischemic stroke population may provide opportunities for future research in the development of novel therapeutic targets for African American and Caucasian ischemic stroke patients.

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