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Original Article

Distinct plasma proteomic changes in male and female African American stroke patients

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Abstract: Background: Stroke occurs more often and results in more severe brain injury in African Americans than in Caucasians. The former also exhibit different responses to thrombolytic therapy than the latter do. There is an imminent need for stroke biomarkers for African Americans, who have been underrepresented in biomarker research for stroke diagnosis and prognosis. Proteomics offers sources for protein biomarkers that are not available by other Omics approaches. In this pilot study, plasma proteomes of African American stroke patients were analyzed and compared to that of hypertensive, non-stroke controls. Methods: Plasma samples were prepared from whole blood specimens that were collected from stroke patients admitted to Grady Memorial Hospital in Atlanta, and their age- and sex-matched, hypertensive controls from the outpatient clinic. Samples were pooled according to patient groups and sex. Plasma proteins were analyzed with quantitative mass spectrometry. The identified and quantified proteins were compared between stroke and control patients of each sex. Proteins that showed changes in abundances in stroke patients were further analyzed with the assistance of bioinformatics tools for their known biological functions or potential implications in stroke. Results: A total of 128 annotated proteins were identified. Results of bioinformatic analysis of plasma proteins whose levels were increased in stroke patients showed, as expected, their association with blood coagulation and inflammation processes. Interestingly, a number of proteins showed different or even opposing changes in male and female stroke patients, notably those involved in IL-4 and IL-6 signaling, complement activation, and blood coagulation disorders. For a few proteins that were increased in female but unchanged or decreased in male stroke patients, an association with fibromuscular dysplasia was recognized. Conclusion: Plasma proteins that differ in quantities between stroke patients and controls were readily detected using a simple proteomic approach. Sex-dependent changes and changes that have not been reported for African American stroke patients offer potentially novel biomarkers for stroke in this underserved population.

Keywords: Stroke, biomarkers, plasma, proteomics, disparity

Introduction

Stroke is the fifth leading cause of death in the United States, killing nearly 130,000 people each year and accounting for approximately one out of every twenty deaths [1]. Stroke risk also varies by race and ethnicity. For African Americans, their risks of having a first stroke are twice as high as that of any other racial groups. Traditional risk factors, such as high blood pressure, obesity and smoking are only partially responsible for an increased risk for stroke [2]. Further, African Americans also have the highest mortality rates after stroke [1]. The determinants for these disparities are poorly understood. In addition, current treatment with recombinant tissue plasminogen activator (rt-PA), the only FDA-approved therapeutics, appears totally ineffective in female African American stroke patients [3]. Accordingly, there is an urgent need to identify biomarkers for stroke in African American populations that not only can assist with stroke diagnosis, but also distinguishes potential differences between male and female stroke patients at molecular levels.

To date, the development of stroke biomarkers for African Americans has been limited. Recently, Meller and colleagues analyzed the transcriptomes of circulating peripheral blood using RNA-Seq technology [4] and reported blood transcriptomic signatures that differentiate,

with high accuracy, stroke patients from controls as well as stroke subtypes among African American stroke patients. While blood transcriptomes are excellent resources for biomarkers with robustness and accuracy, it is understood that proteins are the effectors in establishing cellular functions and properties. Hence, protein analysis is pivotal. Previously, in a study on a small subset of patients from the same cohort of Meller's study, we analyzed the so-called nascent proteomes (proteomes consisting of only newly synthesized proteins within a given time window) of peripheral blood mononuclear cells (PBMC) (Bian et al., 2014) [5]. We found that the PBMC nascent proteomes showed changes that were not seen in the so-called total proteome (all existing proteins disregarding when they were produced by living cells). While Meller's whole blood transcriptomic and our PBMC nascent proteomic studies offer unprecedented accuracy and novelty, respectively, they have limited applicability for other types of biospecimens such as urine, saliva, tears, and plasma, which can be more readily obtained from stroke patients.

The objectives of this study were to examine whether stroke-associated changes in plasma proteomes can be readily detected by a relatively simple proteomic analysis, and to determine whether there are sex-dependent proteomic changes in plasma in African American stroke patients. The study included the same cohort of patients in Bian's study on PBMC nascent proteomes. Our ultimate goal is to expand the development of targeted therapeutics for stroke in African Americans.

Materials and methods

Patient inclusion and demographics

All protocols used in this study were approved by the Institutional Review Board of the Morehouse School of Medicine (MSM) and the Grady Memorial Hospital of Atlanta. Analyses of plasma proteomes were performed on samples from the same African American stroke patients and their age- and gender-matched controls, respectively, that were included in our previous study on PBMC nascent proteomes [5] (n = 5, 4, 3, 3 in male stroke and control, and female stroke and control groups, respectively; average ages were 56, 59, 54, and 54, respectively). The plasma fraction was prepared from whole blood during the process of PBMC isolation using the BD Vacutainer Tubes (BD, Franklin Lakes, NJ). To minimize the interference of high abundant plasma proteins on the detection of those with relative low abundance, the top two most abundant plasma proteins (ALB & IGG) were removed from plasma samples using a commercial kit (SigmaAldrich, St. Louis, MO).

Quantitative mass spectrometry (MS) analysis

Tryptic digests of plasma proteins were prepared following standard protocols and subjected to label-free, quantitative MS analysis [5-7]. The MS system constitutes a nanoflow Ultra Performance Liquid Chromatograph (UPLC) unit (Walters, Milford, MA) that was coupled to a Synapt G2S mass spectrometer (Waters); both were governed by MassLynk software (Waters). Briefly, 0.5-1 μg tryptic peptides, with yeast enolase peptides (50 fmol/μL) mixed in as an internal standard, were fractionated by UPLC through a gradient of 3% to 28% to 40% acetonitrile in 0.1% formic acid over 120 min, at a flow rate of 500 nL/min. A lock mass solution of glu-fibrinopeptide (200 fmol/μL) was applied into the reference sprayer of the NanoLock-Spray source. Electrospray ionization was performed at a potential of 3.0 kV. The mass spectrometer was operated under the HDMSE/RESOLUTION mode with a typical mass resolution of 20,000. Alternating scans were used to detect precursor ions and then fragment ions. The masses of the precursors and the fragment ions were detected with a 0.8 s scan with collision energy (CE) of 4 eV and 19 to 45 eV, respectively. To correct any mass shift that may have had occurred during instrument operation, a single-point calibration was performed against the lock mass compound (doubly-charged glu-fibrinopeptide [mass/charge ratio (m/z) 785.8426]), which was sampled every 30 s. All samples were analyzed with triplicate MS runs.

MS data were analyzed with ProteinLynx Global Server (PLGS) software (Waters, version 2.4). For peptide and protein identification, the MS data were searched against a human protein database downloaded from Uniprot (http://www.uniprot.org) using *homo sapiens* as key word; the database contained 20244 reviewed human protein sequences at the time when the search was performed, and an equal number of reversed sequences. For acceptance, the following rules for data filtering were imposed: at

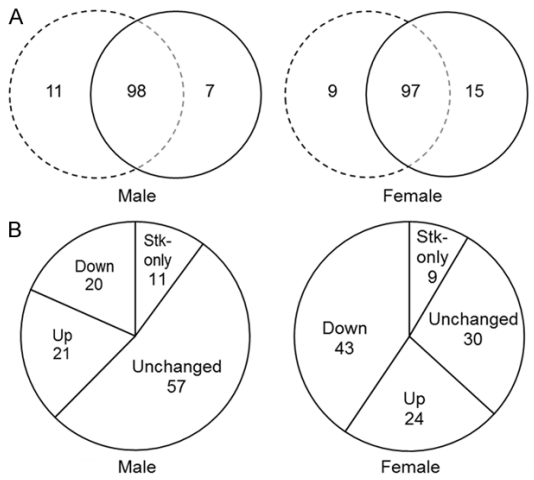

Figure 1. Numbers of proteins identified and quantified. A. Venn diagrams of proteins identified in male (left) and female (right) stroke patients (dashed lines) and their respectively matched controls (solid lines). B. Numbers of proteins that were statistically different in quantities between stroke patients (Stk) and controls (Ctr), or detected only in stroke patients, or showed no changed (unchanged) in stroke patients.

least 1 unique peptide per protein, 3 fragment ions per peptide, 7 fragment ions per protein, mass accuracy less than 10 and 20 parts per million (ppm) of the theoretical masses of precursor and fragment ions, respectively, and a false positive rate less than 4% at protein levels. A protein also must be detected in at least 2 of 3 replicate MS runs. Protein quantitation was achieved by comparing the ion chromatographic peak areas of detected peptides to yeast enolase peptides. The abundances of identified proteins in each sample group were normalized to femtomoles per nanogram to take into account the possible variations in loading amounts of the individual samples. The femtomoles per nanogram values were used to calculate protein ratios between stroke and control groups. Student T test was used to determine the significance of differences in quantities of identified proteins between stroke and control groups.

Bioinformatic analysis

Hierarchical clustering analysis of datasets was performed using Euclidean method with the assistance of the Morpheus software (https://software.broadinstitute.org/morpheus). Proteins that showed significant changes in stroke patients, either increased or decreased when compared with that in controls, were further analyzed with the MetaCore program (Thomson Reuters, Albuquerque, NM), to assess their known function and association with known biological processes.

Results and discussion

Analyzing plasma proteomes with a simple quantitative MS protocol

The publication of a draft human proteome, more than a decade after the publication of human genome [8, 9], has brought-forth the importance and usefulness of proteomics in translational research. Of particular importance is the development of biomarkers in easily accessible bodily fluids, not only for stroke in general, but also for African American stroke patients who respond to rtPA treatment differently [3].

In this pilot study, we used a relatively simple analytical protocol with advanced MS instrumentation, and analyzed pooled plasma samples from both male and female African American stroke patients and controls. A total of 128 annotated proteins were identified and quantified, of which 111 are among the proteins enlisted in the Peptide Atlas for the Human Plasma when queried using the accession numbers (www.peptideatlas.org, 2017 build). Eighty-nine proteins were identified in all 4 pooled samples, whereas the remaining proteins were detected either only in stroke patients or only in controls. The abundances of identified proteins ranged from ng/ml to mg/ml, encompassing three orders of dynamic ranges. The complete list of identified proteins in this study is provided in Table S1 of Supplements. The numbers of proteins that showed a significant difference in abundance between stroke patients and controls are summarized in **Figure 1**.

In reference to the numbers of human plasma proteins that have been documented under the Human Plasma Proteome Project [10], the numbers obtained in this study apparently present only a small fraction of the total plasma proteome. This is not surprising, since the current study used a relatively simple approach (a single depletion step to remove the 2 most abundant proteins and one-dimension pre-MS UPLC peptide separation without any additional

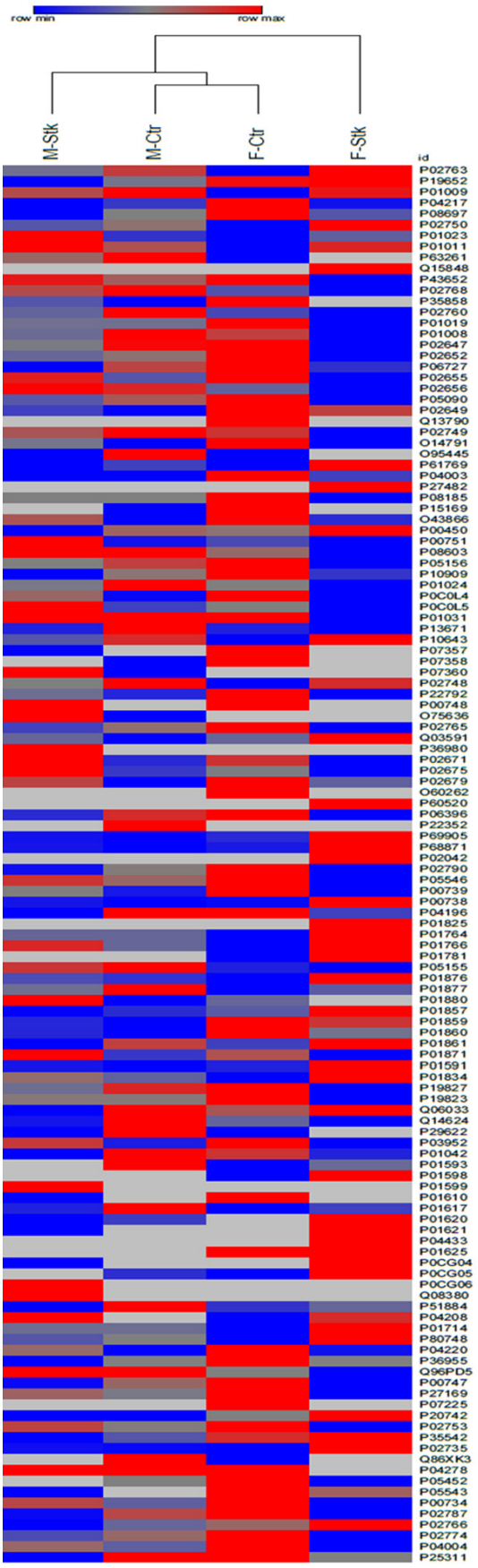

Figure 2. Increased distances between male and female plasma proteomes after stroke. Hierarchical clustering analysis (Euclidean) was performed on the four proteomic datasetsts that were generated from quantitative MS analyses of pooled plasma samples. Stk, stroke; Ctr, control; M and F, male and female, respectively. The colored scale bar indicates abundances of identified proteins (blue, less abundant; red, more abundant; grey, mean abundance).

enrichment steps). In the literature, comparable numbers of plasma proteins have been reported in studies using similar approaches [11], with greater numbers reported in studies involving lengthy MS runs [12] or extensive pre-MS sample processing steps, e.g., depleting additional high-abundant plasma proteins to allow detection of more proteins with relatively low abundances, or fractionating protein preparations into multiple fractions and analyzing those fractions individually [13]. Using a simple protocol allowed us to minimize technical variations that could be introduced by including additional analytical steps, achieving an average of relative standard deviation (RSD) of 0.11 in triplicate quantitative MS analyses of all samples. With the limit in both patient numbers and numbers of identified plasma proteins, we caution not to make definite conclusions in regard to potential biomarkers for stroke in African Americans. Rather, we attempted to evaluate whether or not the current data are indicative of sex-specific changes in plasma proteomes in stroke.

Distinct changes in plasma proteomes in male and female stroke patients

To compare the similarity, or the lack of it, of plasma proteomes of the four study groups, namely male stroke and control, and female stroke and control (M-Stk, M-Ctr, F-Stk, F-Ctr, respectively), proteomic datasets were subjected to hierarchical clustering analysis. As shown in **Figure 2**, among groups, the closest distance was observed between male and female controls, whereas the greatest distance was seen between male and female stroke patients. Similar results of hierarchical clustering have been seen in our previous studies on PBMC proteomes in African American stroke patients [5]. This implicates that, while the plasma (or blood) proteomes are expected to be different between male and female in non-stroke conditions, the differences are further marked after stroke. In other words, the plasma proteomic changes after a stroke could be sex-dependent.

Table 1. Enrichment analysis of proteins that showed an increase in stroke patients

Biological Processes	
Male	Female
Protein activation cascade	Complement activation, classical pathway
Complement activation	Protein activation cascade
Defense response	Humoral immune response mediated by circulating immunoglobulin
Humoral immune response	Complement activation
Immune system process	Humoral immune response
Complement activation, classical pathway	Immunoglobulin mediated immune response
Immune response	B cell mediated immunity
Humoral immune response mediated by circulating immunoglobulin	Lymphocyte mediated immunity
Response to stress	Immune system process
Immunoglobulin mediated immune response	Defense response
Process Networks	
Male	Female
Inflammation_Kallikrein-kinin system	Inflammation_IL-6 signaling
Blood coagulation	Immune response_BCR pathway
Inflammation_IL-6 signaling	Blood coagulation
Inflammation_Complement system	Immune response_Phagocytosis
Proteolysis_ECM remodeling	Inflammation_Kallikrein-kinin system
Cell adhesion_Platelet-endothelium-leucocyte interactions	Inflammation_Histamine signaling
Signal transduction_Leptin signaling	Proteolysis_ECM remodeling
Cell adhesion_Integrin priming	Immune response_Phagosome in antigen presentation
Proteolysis_Connective tissue degradation	Signal transduction_Leptin signaling
Development_Hemopoiesis, Erythropoietin pathway	Inflammation_IL-4 signaling
Diseases	
Male	Female
Blood Coagulation Disorders	Amyloidosis
Hemorrhage	Cystic Fibrosis
Thrombosis	Infant, Newborn, Diseases
Embolism and Thrombosis	Arthritis
Capillary Leak Syndrome	Joint Diseases
Myocardial Ischemia	Keratoconjunctivitis
Chest Pain	Proteostasis Deficiencies
Angina Pectoris	Multiple Myeloma
Vascular Diseases	Conjunctivitis
Hemorrhagic Disorders	Paraproteinemias

Enrichment Analysis was performed for proteins that were increased in plasma in stroke patients. The lists include the top-ten most significant processes or networks that are associated with changed proteins, as determined by the MetaCore program.

With the assistance of the MetaCore program, we assessed the association of proteins that were increased in stroke patients with known biological processes or signaling pathways. As shown in **Table 1**, not surprisingly, in both male and female stroke patients, the up-regulated plasma proteins were predominantly those involved in immune response and blood coagulation. Interestingly, when proteins that were increased in male stroke patients were compared with that in female stroke patients, there were noticeable differences (**Table 1**; **Figure 3**). For example, the increase in proteins involved in IL-4 or IL-6 signaling processes was more prominent in female than in male stroke patients, whereas the increase in Kallikrein-kinin signaling or classical complement pathways were more prominent in male than in female stroke patients.

Roles of proinflammatory cytokines in stroke and many other diseases have been studied intensively. It is known that profiles of cytokines, including IL-4 and IL-6, exhibit population- and sex-associated differences under normal conditions as well as in response to stresses [14-18]. Serum levels of IL-6 have been shown to correlate with severity of brain injury in stroke [14, 19]; IL-4 may play a role in sex-dependent vulnerability to brain injury in an ani-

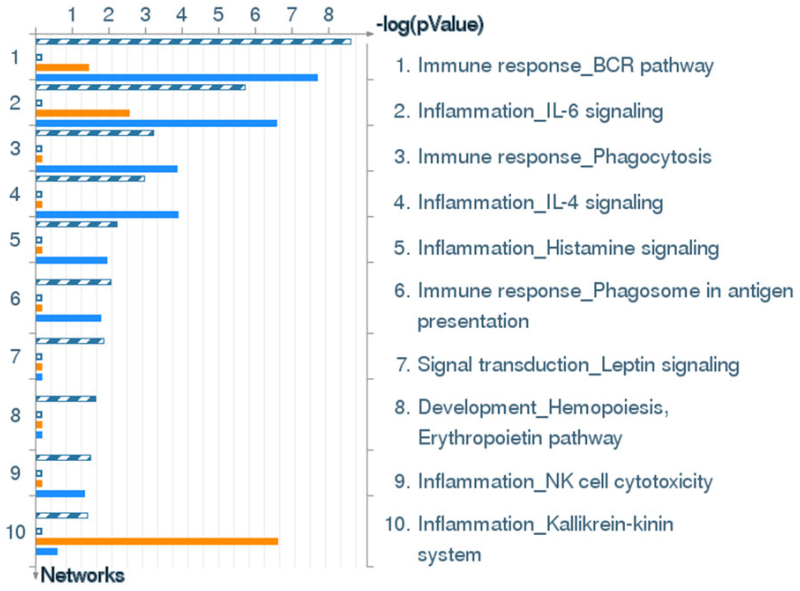

Figure 3. Comparison of Process Networks that were enriched in male and female stroke patients. The figure shows levels of significance in enrichment in noted processes that are associated with proteins that were increased in male (orange) or female (blue) stroke patients, as determined by the Enrichment Analysis using the MetaCore program.

mal model of focal cerebral ischemia [20]. Kallikrein-kinin signaling pathways or complement levels have been shown to play regulatory roles in stroke or cardiovascular diseases in general, in human studies or studies using animal models [21-23]. Worth-noting is that little is known regarding how plasma levels of proteins involved in the afore-noted mechanisms may affect a stroke patient's response to rtPA therapy in sex-specific manners, and less about the question of their contribution to the efficacy of rtPA therapy in African American stroke patients, given that current literature is primarily limited to studies on Caucasian populations [24].

Further, we analyzed proteins that showed changes in opposite directions in male and female stroke patients. As shown in **Table 2**, different diseases or disorder conditions, for which there are existing biomarkers in literature, were recognized with such proteins in male and female stroke patients. For example, in male stroke patients, the up-regulated proteins included those that have been indicated in thrombophilia or hemorrhage conditions, whereas in female stroke patients, fibromuscular dysplasia (FMD) was recognized with up-regulated proteins. The latter condition is known to be a stroke risk factor, more common in women (FMD Fact Sheet, www.fmdsa.org), and has different arterial involvement in men and women [25]. Little is known whether African American women may be more prone to develop FMD than men or other populations are. It also remains to be examined whether after a stroke people with FMD may respond to rtPA treatment differently than those without do.

A further examination of individual proteins that contributed to the above-mentioned, sex-dependent proteomic changes in the plasma identified a number of proteins of interests. Among them are immunoglobulin heavy constant gamma 1 (IGHG1) and alpha-1-acid glycoprotein 1 (ORM1) proteins which showed an increase in female patients but a decrease or no change in male stroke patients. IGHG1 is an antigen and an immunoglobulin binding protein. It is known to be involved in B-cell receptor signaling pathways and acts as a positive regulator of B-cell activation. Though IGHG1 has not been considered as a stroke biomarker, it has been implicated in the pathogenesis of prostate cancer [26] and Alzheimer's disease [7]. ORM1 is a transport protein involved in regulating the immune system. In postmenopausal women, ORM1 may have the potential as a risk marker for chronic heart disease [27]. Gene transcript levels of ORM1 have been shown to increase in stroke patients [28]. Our results, if confirmed in future analyses on larger patient cohorts, may warrant IGHG1 and ORM1 for further studies for their potentials as biomarkers that can differentiate male and female stroke patients, especially for African Americans.

Taken together, the current results demonstrate that proteomics can be an effective approach in revealing sex-specific changes in plasma proteomes after a stroke.

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Table 2. Enrichment analysis of proteins that showed opposing changes in male and female stroke patients

Biological Processes	
M, Up/F, Down	F, Up/M, Down
Protein activation cascade	Leukocyte mediated immunity
Fibrinolysis	Response to bacterium
Blood coagulation, fibrin clot formation	Immune effector process
Defense response	Response to other organism
Humoral immune response	Response to external biotic stimulus
Iinnate immune response	Response to biotic stimulus
Regulation of protein activation cascade	Acute-phase response
Negative regulation of hemostasis	Defense response
Negative regulation of blood coagulation	Vesicle-mediated transport
Negative regulation of coagulation	Immune response
Process Networks	
M, Up/F, Down	F, Up/M, Down
Blood coagulation	Inflammation_IL-6 signaling
Inflammation_Kallikrein-kinin system	Proteolysis_ECM remodeling
Inflammation_Complement system	Blood coagulation
Proteolysis_ECM remodeling	Proteolysis_Connective tissue degradation
Inflammation_IL-6 signaling	Inflammation_Kallikrein-kinin system
Cell adhesion_Platelet-endothelium-leucocyte interactions	
Cell adhesion_Integrin priming	
Cell adhesion_Platelet aggregation	
Signal transduction_CREM pathway	
Inflammation_Protein C signaling	
Diseases	
M, Up/F, Down	F, Up/M, Down
Thrombophilia	Fibromuscular Dysplasia
Blood Coagulation Disorders	Escherichia coli Infections
Myocardial Infarction	Alcohol-Related Disorders
Hemorrhage	Asbestosis
Purpura, Thrombotic Thrombocytopenic	Esophageal and Gastric Varices
Thrombosis	Subcutaneous Emphysema
Embolism and Thrombosis	alpha 1-Antitrypsin Deficiency
Thrombocytopenia	Mucopolysaccharidosis IV
Chest Pain	Substance-Related Disorders
Angina Pectoris	Hypertension, Portal

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Disclosure of conflict of interest

None.

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Table S1. Identification and quantitation of plasma proteins from African American stroke patients

Protein accession No	Protein Description	F Stk			F Ctr			t test	Stk Vs Ctr	M Stk			M Ctr			t test	Stk vs Ctr
		Mean	Std	RSD	Mean	Std	RSD			Mean	Std	RSD	Mean	Std	RSD		
P02763	Alpha 1 acid glycoprotein 1	0.68	0.02	0.03	0.40	0.01	0.03	0.00		0.53	0.03	0.07	0.62	0.01	0.01	0.01	-
P19652	Alpha 1 acid glycoprotein 2	0.12	0.00	0.00	0.12	0.00	0.03	0.47		0.07	0.01	0.19	0.09	0.03	0.34	0.38	
P01009	Alpha 1 antitrypsin	1.46	0.04	0.03	0.91	0.04	0.04	0.00		1.33	0.06	0.05	1.51	0.08	0.05	0.04	-
P04217	Alpha 1B glycoprotein	0.10	0.00	0.03	0.13	0.01	0.05	0.00	-	0.09	0.00	0.03	0.10	0.00	0.01	0.02	-
P08697	Alpha 2 antiplasmin	0.03	0.00	0.05	0.04	0.00	0.01	0.00	-	0.03	0.00	0.06	0.04	0.00	0.08	0.03	-
P02750	Leucine rich alpha 2 glycoprotein	0.02	0.00	0.03	0.01	0.00	0.08	0.00		0.02	0.00	0.06	0.02	0.00	0.11	0.16	
P01023	Alpha 2 macroglobulin	0.61	0.01	0.01	0.53	0.02	0.04	0.00		0.76	0.04	0.05	0.57	0.01	0.02	0.00	
P01011	Alpha 1 antichymotrypsin	0.14	0.01	0.04	0.09	0.00	0.02	0.00		0.15	0.00	0.02	0.13	0.01	0.08	0.03	
P63261	Actin cytoplasmic 2				0.01	0.00	0.08		Ctr	0.02	0.01	0.81	0.02	0.00	0.03	0.59	
Q15848	Adiponectin	0.02	0.01	0.46					Stk								
P43652	Afamin	0.02	0.00	0.08	0.03	0.00	0.02	0.00	-	0.03	0.00	0.03	0.03	0.00	0.00	0.01	
P02768	Serum albumin	0.07	0.00	0.02	0.08	0.00	0.02	0.00	-	0.09	0.01	0.07	0.10	0.00	0.02	0.06	
P35858	Insulin like growth factor binding protein complex acid labile subunit				0.01	0.00	0.16		Ctr	0.01	0.00	0.00	0.00	0.00	0.00		
P02760	Protein AMBP	0.06	0.01	0.13	0.07	0.01	0.10	0.48		0.07	0.01	0.17	0.08	0.01	0.11	0.26	
P01019	Angiotensinogen	0.03	0.00	0.02	0.06	0.00	0.04	0.00	-	0.04	0.00	0.05	0.04	0.00	0.05	0.71	
P01008	Antithrombin III	0.05	0.00	0.02	0.06	0.00	0.04	0.00	-	0.05	0.00	0.03	0.06	0.00	0.05	0.02	-
P02647	Apolipoprotein A I	0.94	0.04	0.04	2.85	0.08	0.03	0.00	-	1.86	0.01	0.01	2.79	0.19	0.07	0.00	-
P02652	Apolipoprotein A II SV 1	0.14	0.05	0.36	0.50	0.05	0.11	0.00	-	0.28	0.11	0.39	0.34	0.13	0.37	0.59	
P06727	Apolipoprotein A IV	0.05	0.00	0.03	0.09	0.00	0.02	0.00	-	0.04	0.00	0.04	0.08	0.00	0.05	0.00	-
P02655	Apolipoprotein C II	0.02	0.01	0.23	0.03	0.00	0.03	0.06		0.03	0.00	0.14	0.03	0.01	0.39	0.47	
P02656	Apolipoprotein C III	0.07	0.00	0.03	0.08	0.01	0.18	0.40		0.09	0.00	0.04	0.09	0.00	0.04	0.39	
P05090	Apolipoprotein D	0.04	0.00	0.03	0.07	0.00	0.06	0.00	-	0.05	0.00	0.06	0.06	0.01	0.11	0.08	
P02649	Apolipoprotein E	0.04	0.00	0.04	0.04	0.00	0.03	0.29		0.04	0.00	0.03	0.04	0.00	0.02	0.07	
Q13790	Apolipoprotein F				0.01	0.00	0.32		Ctr								
P02749	Beta 2 glycoprotein 1	0.10	0.00	0.03	0.13	0.01	0.06	0.01	-	0.12	0.00	0.02	0.13	0.00	0.02	0.02	-
014791	Apolipoprotein L1	0.01	0.00	0.10	0.02	0.00	0.07	0.00	-	0.02	0.00	0.10	0.01	0.00	0.20	0.02	
095445	Apolipoprotein M				0.01	0.00	0.09		Ctr	0.01	0.00	0.00	0.01	0.00	0.20	0.42	
P61769	Beta 2 microglobulin	0.02	0.00	0.06	0.01	0.00	0.06	0.00		0.01	0.00	0.07	0.01	0.00	0.14	0.16	
P04003	C4b binding protein alpha chain	0.01	0.00	0.07	0.01	0.00	0.08	0.02	-	0.01	0.00	0.11	0.01	0.00	0.00	1.00	
P27482	Calmodulin like protein 3	0.00	0.00	0.24					Stk								
P08185	Corticosteroid binding globulin	0.02	0.00	0.07	0.02	0.00	0.08	0.07		0.02	0.00	0.03	0.02	0.00	0.07	0.42	
P15169	Carboxypeptidase N catalytic chain				0.01	0.00	0.00		Ctr				0.00	0.00	0.00		Ctr
043866	CD5 antigen like	0.01	0.00	0.17	0.02	0.00	0.00	0.01	-	0.01	0.00	0.00	0.01	0.00	0.07	0.00	
P00450	Ceruloplasmin	0.22	0.01	0.04	0.21	0.00	0.02	0.07		0.19	0.01	0.07	0.21	0.00	0.02	0.07	
P00751	Complement factor B	0.09	0.00	0.04	0.09	0.00	0.03	0.14		0.10	0.00	0.04	0.09	0.00	0.01	0.00	
P08603	Complement factor H	0.05	0.00	0.02	0.06	0.00	0.04	0.00	-	0.07	0.00	0.01	0.07	0.00	0.02	1.00	
P05156	Complement factor I	0.01	0.00	0.12	0.02	0.00	0.20	0.10		0.02	0.00	0.04	0.02	0.00	0.13	0.47	

P10909	Clusterin	0.08	0.00	0.02	0.09	0.00	0.03	0.00	-	0.07	0.00	0.03	0.08	0.00	0.02	0.00	-
P01024	Complement C3	0.38	0.01	0.02	0.40	0.02	0.05	0.08		0.40	0.02	0.04	0.43	0.02	0.04	0.09	
P0C0L4	Complement C4 A	0.03	0.00	0.05	0.08	0.00	0.01	0.00	-	0.06	0.00	0.06	0.03	0.00	0.07	0.00	
P0C0L5	Complement C4 B	0.07	0.00	0.02	0.08	0.00	0.03	0.00	-	0.09	0.00	0.04	0.07	0.00	0.06	0.00	
P01031	Complement C5	0.01	0.00	0.00	0.01	0.00	0.19	0.26		0.01	0.00	0.04	0.01	0.00	0.04	1.00	
P13671	Complement component C6	0.01	0.00	0.13	0.01	0.00	0.00	0.12		0.01	0.00	0.07	0.02	0.02	0.92	0.56	
P10643	Complement component C7	0.02	0.01	0.41	0.01	0.00	0.04	0.28		0.01	0.00	0.16	0.02	0.00	0.00	0.07	
P07357	Complement component C8 alpha chain				0.01	0.00	0.07		Ctr	0.01	0.00	0.00					Stk
P07358	Complement component C8 beta chain				0.01	0.00	0.33		Ctr				0.01	0.00	0.20		Ctr
P07360	Complement component C8 gamma chain									0.01	0.00	0.09	0.01	0.00	0.55	0.96	
P02748	Complement component C9	0.02	0.00	0.08	0.02	0.00	0.03	0.01		0.02	0.00	0.16	0.02	0.00	0.06	0.25	
P22792	Carboxypeptidase N subunit 2	0.01	0.00	0.06	0.02	0.00	0.00	0.00	-	0.01	0.00	0.00	0.01	0.00	0.24	0.16	
P00748	Coagulation factor XII				0.01	0.00	0.00		Ctr	0.01	0.00	0.00					Stk
075636	Ficolin 3									0.01	0.00	0.18	0.01	0.00	0.11	0.31	
P02765	Alpha 2 HS glycoprotein	0.12	0.01	0.06	0.20	0.01	0.03	0.00	-	0.14	0.00	0.02	0.17	0.01	0.03	0.00	-
Q03591	Complement factor H related protein 1	0.01	0.01	0.78	0.01	0.00	0.23	0.51		0.01	0.00	0.25	0.01	0.00	0.53	0.33	
P36980	Complement factor H related protein 2									0.00	0.00	0.28					Stk
P02671	Fibrinogen alpha chain	0.30	0.01	0.03	0.39	0.01	0.01	0.00	-	0.42	0.02	0.04	0.32	0.01	0.04	0.00	
P02675	Fibrinogen beta chain	0.45	0.02	0.04	0.52	0.01	0.03	0.01	-	0.60	0.06	0.10	0.48	0.02	0.05	0.04	
P02679	Fibrinogen gamma chain	0.98	0.03	0.03	1.11	0.03	0.02	0.00	-	1.06	0.04	0.04	0.90	0.05	0.06	0.02	
060262	Guanine nucleotide binding protein GIGSGO subunit gamma 7				0.00	0.00	0.20	Ctr									
P60520	Gamma aminobutyric acid receptor associated protein like 2	0.08	0.05	0.68					Stk								
P06396	Gelsolin	0.02	0.00	0.07	0.03	0.00	0.06	0.01	-	0.02	0.00	0.05	0.03	0.00	0.04	0.02	-
P22352	Glutathione peroxidase 3												0.01	0.00	0.00		Ctr
P69905	Hemoglobin subunit alpha	0.17	0.00	0.02	0.06	0.00	0.04	0.00		0.05	0.00	0.09	0.04	0.00	0.07	0.03	
P68871	Hemoglobin subunit beta	0.16	0.01	0.06	0.05	0.00	0.02	0.00		0.05	0.00	0.05	0.04	0.00	0.06	0.01	
P02042	Hemoglobin subunit delta	0.01	0.00	0.14					Stk								
P02790	Hemopexin	0.72	0.04	0.06	0.92	0.02	0.02	0.00	-	0.73	0.05	0.06	0.82	0.06	0.07	0.10	
P05546	Heparin cofactor 2	0.02	0.00	0.05	0.03	0.00	0.06	0.01	-	0.02	0.00	0.05	0.02	0.00	0.04	0.49	
P00739	Haptoglobin related protein	0.05	0.00	0.02	0.11	0.00	0.03	0.00	-	0.08	0.00	0.03	0.06	0.00	0.01	0.00	
P00738	Haptoglobin	2.27	0.06	0.03	1.55	0.07	0.05	0.00		1.58	0.06	0.03	1.52	0.13	0.08	0.49	
P04196	Histidine rich glycoprotein	0.04	0.00	0.00	0.05	0.00	0.03	0.00	-	0.03	0.00	0.02	0.05	0.00	0.01	0.00	-
P01825	Ig heavy chain V II region NEWM	0.02	0.00	0.15					Stk								
P01764	Ig heavy chain V III region VH26	0.02	0.01	0.31	0.01	0.00	0.27	0.03		0.01	0.00	0.07	0.01	0.00	0.08	0.72	
P01766	Ig heavy chain V III region BRO	0.05	0.01	0.25	0.03	0.00	0.02	0.12		0.05	0.01	0.15	0.04	0.01	0.16	0.23	
P01781	Ig heavy chain V III region GAL	0.02	0.00	0.15	0.01	0.00	0.17	0.03									
P05155	Plasma protease C1 inhibitor	0.08	0.00	0.01	0.09	0.00	0.03	0.07		0.11	0.00	0.02	0.12	0.01	0.08	0.32	
P01876	Ig alpha 1 chain C region	2.43	0.05	0.02	0.78	0.03	0.04	0.00		1.27	0.08	0.06	1.10	0.03	0.03	0.02	
P01877	Ig alpha 2 chain C region	0.10	0.01	0.07	0.04	0.01	0.13	0.00		0.11	0.01	0.07	0.19	0.00	0.01	0.00	-

P01880	Ig delta chain C region				0.01	0.00	0.23		Ctr	0.02	0.00	0.25	0.01	0.00	0.38	0.03	
P01857	Ig gamma 1 chain C region	0.14	0.00	0.03	0.10	0.00	0.05	0.00		0.08	0.01	0.14	0.09	0.01	0.12	0.34	
P01859	Ig gamma 2 chain C region	0.05	0.00	0.06	0.05	0.00	0.09	0.08		0.02	0.00	0.11	0.02	0.00	0.16	0.11	
P01860	Ig gamma 3 chain C region	0.02	0.00	0.11	0.03	0.00	0.02	0.00	-	0.01	0.00	0.22	0.01	0.00	0.36	0.40	
P01861	Ig gamma 4 chain C region	0.01	0.00	0.38	0.01	0.00	0.54	0.43		0.01	0.01	1.21	0.01	0.01	1.12	0.75	
P01871	Ig mu chain C region3	0.07	0.01	0.11	0.12	0.01	0.07	0.00	-	0.14	0.02	0.12	0.08	0.01	0.17	0.01	
P01591	Immunoglobulin J chain	0.04	0.00	0.04	0.02	0.00	0.20	0.00		0.02	0.00	0.00	0.02	0.00	0.04	0.01	
P01834	Ig kappa chain C region	1.18	0.10	0.09	0.51	0.01	0.01	0.00		0.89	0.06	0.07	0.76	0.04	0.05	0.03	
P19827	Inter alpha trypsin inhibitor heavy chain H1	0.05	0.00	0.03	0.07	0.01	0.07	0.00	-	0.06	0.00	0.04	0.07	0.00	0.06	0.04	-
P19823	Inter alpha trypsin inhibitor heavy chain H2	0.07	0.00	0.04	0.13	0.01	0.06	0.00	-	0.10	0.02	0.18	0.10	0.00	0.01	0.98	
Q06033	Inter alpha trypsin inhibitor heavy chain H3	0.01	0.00	0.00	0.01	0.00	0.44	0.65		0.01	0.00	0.08	0.01	0.00	0.19	0.12	
Q14624	Inter alpha trypsin inhibitor heavy chain H4	0.04	0.00	0.02	0.05	0.00	0.01	0.00	-	0.05	0.00	0.04	0.06	0.00	0.04	0.00	-
P29622	Kallistatin				0.01	0.00	0.00		Ctr	0.01	0.00	0.08	0.01	0.00	0.25	0.54	
P03952	Plasma kallikrein	0.01	0.00	0.13	0.02	0.01	0.49	0.14		0.02	0.01	0.47	0.01	0.00	0.11	0.22	
P01042	Kininogen 1	0.12	0.00	0.02	0.13	0.00	0.03	0.02	-	0.12	0.00	0.01	0.13	0.00	0.03	0.01	-
P01593	Ig kappa chain V I region AG	0.03	0.00	0.03	0.02	0.00	0.24	0.01					0.05	0.00	0.08		Ctr
P01598	Ig kappa chain V I region EU	0.01	0.01	0.45	0.01	0.00	0.16	0.97									
P01599	Ig kappa chain V I region Gal									0.02	0.00	0.23					Stk
P01610	Ig kappa chain V I region WEA				0.02	0.01	0.48		Ctr	0.01	0.01	0.68					Stk
P01617	Ig kappa chain V II region TEW	0.03	0.01	0.34	0.02	0.00	0.02	0.72		0.03	0.01	0.35	0.03	0.01	0.30	0.41	
P01620	Ig kappa chain V III region SIE	0.06	0.00	0.03					Stk	0.04	0.00	0.08	0.05	0.01	0.21	0.36	
P01621	Ig kappa chain V III region NG9 Fragment	0.03	0.01	0.34					Stk	0.01	0.00	0.14					Stk
P04433	Ig kappa chain V III region VG Fragment	0.02	0.00	0.18					Stk								
P01625	Ig kappa chain V IV region Len	0.01	0.00	0.10	0.01	0.00	0.05	0.42									
P0CG04	Ig lambda 1 chain C regions	0.15	0.04	0.27					Stk	0.08	0.03	0.34					Stk
P0CG05	Ig lambda 2 chain C regions	0.80	0.08	0.10	0.28	0.02	0.06	0.00					0.38	0.01	0.04		Ctr
P0CG06	Ig lambda 3 chain C regions									0.34	0.03	0.09					Stk
Q08380	Galectin 3 binding protein									0.01	0.00	0.07					Stk
P51884	Lumican	0.02	0.00	0.04	0.01	0.00	0.08	0.09		0.01	0.00	0.13	0.02	0.00	0.06	0.01	-
P04208	Ig lambda chain V I region WAH	0.01	0.00	0.12	0.01	0.00	0.00	0.01		0.01	0.00	0.00					Stk
P01714	Ig lambda chain V III region SH	0.01	0.00	0.05	0.01	0.00	0.00	0.00		0.01	0.00	0.08	0.01	0.00	0.09	0.79	
P80748	Ig lambda chain V III region LOI	0.03	0.00	0.06	0.01	0.00	0.09	0.00		0.02	0.00	0.05	0.02	0.00	0.05	0.02	-
P04220	Ig mu heavy chain disease protein	0.04	0.01	0.23	0.08	0.01	0.18	0.01	-	0.06	0.02	0.32	0.03	0.01	0.24	0.10	
P36955	Pigment epithelium derived factor	0.01	0.00	0.05	0.01	0.00	0.05	0.52		0.01	0.00	0.13	0.01	0.00	0.05	0.35	
Q96PD5	N acetylmuramoyl L alanine amidase	0.01	0.00	0.05	0.01	0.00	0.04	0.01	-	0.02	0.00	0.04	0.02	0.00	0.04	1.00	
P00747	Plasminogen	0.05	0.00	0.07	0.08	0.02	0.24	0.07		0.05	0.01	0.09	0.07	0.00	0.05	0.01	-
P27169	Serum paraoxonase arylesterase 1	0.04	0.00	0.01	0.06	0.00	0.00	0.00	-	0.05	0.00	0.04	0.05	0.00	0.08	0.27	
P07225	Vitamin K dependent protein S				0.01	0.00	0.37		Ctr								
P20742	Pregnancy zone protein	0.01	0.00	0.08	0.01	0.00	0.64	0.69		0.01	0.00	0.20	0.01	0.00	0.28	1.00	
P02753	Retinol binding protein 4	0.02	0.00	0.00	0.05	0.00	0.03	0.00	-	0.04	0.00	0.01	0.04	0.00	0.02	0.00	
P35542	Serum amyloid A 4 protein	0.02	0.00	0.00	0.02	0.01	0.23	0.76		0.02	0.00	0.08	0.02	0.01	0.33	0.64	

P02735	Serum amyloid A protein	0.08	0.00	0.06	0.00	0.00	0.12	0.00		0.01	0.00	0.18	0.01	0.00	0.25	0.28	
Q86XK3	Swi5 dependent recombination DNA repair protein 1 homolog				0.01	0.00	0.09		Ctr				0.01	0.01	0.51	Ctr	
P04278	Sex hormone binding globulin				0.00	0.00	0.00		Ctr	0.00	0.00	0.00	0.00	0.00	0.00	1.00	
P05452	Tetranectin	0.01	0.00	0.19	0.01	0.00	0.00	0.07					0.01	0.00	0.08		Ctr
P05543	Thyroxine binding globulin	0.01	0.01	0.69	0.02	0.01	0.78	0.70		0.01	0.00	0.00					Stk
P00734	Prothrombin	0.06	0.00	0.07	0.10	0.01	0.05	0.00	-	0.09	0.00	0.04	0.08	0.00	0.03	0.00	
P02787	Serotransferrin	1.19	0.07	0.06	1.61	0.02	0.01	0.00	-	1.20	0.08	0.07	1.49	0.03	0.02	0.00	-
P02766	Transthyretin	0.03	0.00	0.04	0.02	0.00	0.02	0.02		0.02	0.00	0.09	0.02	0.00	0.13	0.40	
P02774	Vitamin D binding protein	0.19	0.01	0.07	0.24	0.01	0.03	0.00	-	0.21	0.00	0.02	0.22	0.02	0.09	0.27	
P04004	Vitronectin OS PE 1 SV 1	0.06	0.00	0.02	0.08	0.00	0.02	0.00	-	0.07	0.01	0.07	0.07	0.01	0.13	0.39	
P25311	Zinc alpha 2 glycoprotein	0.03	0.00	0.00	0.03	0.00	0.02	0.02	-	0.03	0.00	0.04	0.03	0.00	0.06	0.24	
Mean RSD				0.107			0.098					0.110			0.123		

Plasma samples were pooled according to patient groups. Each pooled samples were analyzed in triplicates with quantitative MS. Changed proteins include proteins that showed statistically significant differences in abundance (fmol/ng) in comparisons between stroke (Stk) and control (Ctr) groups, and those that were identified only in Stk or Ctr groups. F and M, female and male, respectively; RSD: Relative Standard Deviation.