readthrough: 24 hours before transfection, HEK293 cells were split in 6-well plates. On the following day, approximately 60% confluence, the cells were transiently transfected with the WT or PTC mutated constructs using Polyethylenimine HCl MAX. Cells were transfected with a total amount of 0.35 µg DNA/well and 2 µl Polyethylenimine HCl MAX/well. Four hours later, the transfection medium was removed and replaced with fresh medium, without streptomycin and penicillin. The fresh media contained gentamicin diluted to the indicated concentration per well. Fresh gentamicin-containing medium was replaced after 24 hours. After 48 hours, lysates were collected in 100 µL mRIPA supplemented with protease inhibitors for each construct. The lysates were run on a western blot and the N-terminal was probed with anti-FLAG. A malachite green phosphatase assay to measure inorganic phosphate release from phospho-glucans, that is glycogen or LBs. Glycogen is used in this laforin bioassay as the biologically relevant substrate in order to determine the specific activity of the readthrough products. All reactions are incubated for 40 minute the absorbance is measured at 620 nm and the pmoles of phosphate released/min/nmol protein was calculated using a standard curve. RESULTS/ANTICIPATED RESULTS: HEK293 cells were transfected with MeCP2 R241X, laforin R241X, or laforin WT NT-FLAG construct, treated with different concentrations of gentamicin for 48 hours, and laforin levels were assessed by Western analysis with anti-FLAG. HEK293 cells were transfected with WT laforin or a laforin PTC CT-FLAG construct, treated with different concentrations of gentamicin for 48 hours, and laforin levels were assessed by Western analysis with anti-FLAG. B. Quantification of read-through for PTC experiments. *p-value ≤ 0.001. #p-value ≤ 0.001. Schematic of laforin bioassay. The assay has been performed with human and mouse tissue as well as cultured cells. B. Laforin bioassay results using laforin from PTC experiment. **p-value ≤ 0.001. *p-value ≤ 0.01. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results suggest that gentamicin is not only responsible for inducing readthrough of the PTC mutations, but also for promoting translation of fully functional laforin. Therefore, our in vitro system for the analysis of PTC readthrough of laforin will be useful for determining which PTC mutations are suppressible with gentamicin or other small molecules, in what quantities laforin is recovered from PTC mutations, and if the protein products possess the appropriate enzymatic function.

2357

Lost and found: Detection of brain cardiolipins in plasma after cardiac arrest

Andrew M. Lamade[1,2], Tamil S. Anthonymuthu[1,2], Elizabeth M. Kenny[1,2], Hitesh Gidwani[1], Nicholas M. Krehel[1], Andrew A. Amoscato[2], Adam C. Straub[3,4], Valerian E. Kagan[2], Cameron Dezfulian[1,4] and Hülya Bayır[1,2]
[1] Department of Critical Care Medicine, School of Medicine, Safar Center for Resuscitation Research, University of Pittsburgh and Children's Hospital of Pittsburgh, Pittsburgh, PA, USA; [2] Center for Free Radical and Antioxidant Health, Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA; [3] Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA; [4] Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, PA, USA

OBJECTIVES/SPECIFIC AIMS: Neurological injury remains as the main limiting factor for overall recovery after cardiac arrest (CA). Currently available indicators of neurological injury are inadequate for early prognostication after return of spontaneous circulation (ROSC). High diversification of brain mitochondrial cardiolipins (CL) makes them unique candidates to quantify brain injury and to predict prognosis early after ROSC. METHODS/STUDY POPULATION: CL content in plasma in 39 patients within 6 hours of ROSC and 10 healthy subjects as well as CL content in human heart and brain specimens were quantified using a high-resolution liquid chromatography mass spectrometry method. The quantities of brain-type CL species were correlated with clinical parameters of brain injury severity permitting derivation of a cerebral CL score (C-score) using linear regression. C-score and a single CL species (70:5) were evaluated in patients with varying neurological injury and outcome. Using a rat model of CA, CL was quantified in the plasma and brain of rats using similar methods and results compared with the controls. RESULTS/ANTICIPATED RESULTS: We found that brain and the heart fell on extreme ends of the CL diversity spectrum with 26 species of CL exclusively present in human brain not heart. Nine of these 26 species were present in plasma within 6 hours of ROSC with quantities correlating with greater brain injury. The C-score correlated with early neurologic injury and predicted discharge neurologic/functional outcome. CL (70:5) emerged as a

potential point-of-care marker that alone was predictive of injury severity and outcome nearly as well as C-score. Using a rat CA model we showed a significant reduction in hippocampal CL content corresponding to CL released from the brain into systemic circulation. C-score was significantly increased in 10 minute Versus 5 minute no-flow CA and naïve controls. DISCUSSION/SIGNIFICANCE OF IMPACT: CA results in appearance and accumulation of CL in plasma, proportional to injury severity. Quantitation of brain-type CL species in plasma can be used to prognosticate neurological injury within 6 hours after ROSC.

2190

Longitudinal changes in EEG power envelope connectivity are proportional to motor recovery in chronic stroke patients

Joseph B. Humphries[1], David T. Bundy[2], Eric C. Leuthardt[3] and Thy N. Huskey[3]
[1] Institute of Clinical and Translational Sciences, Washington University in St. Louis; [2] Post-Doctoral Trainee, University of Kansas Medical Center; [3] Mid-Career Investigator, Washington University School of Medicine in St. Louis

OBJECTIVES/SPECIFIC AIMS: The objective of this study is to determine the degree to which the use of a contralesionally-controlled brain-computer interface for stroke rehabilitation drives change in interhemispheric motor cortical activity. METHODS/STUDY POPULATION: Ten chronic stroke patients were trained in the use of a brain-computer interface device for stroke recovery. Patients perform motor imagery to control the opening and closing of a motorized hand orthosis. This device was sent home with patients for 12 weeks, and patients were asked to use the device 1 hour per day, 5 days per week. The Action Research Arm Test (ARAT) was performed at 2-week intervals to assess motor function improvement. Before the active motor imagery task, patients were asked to quietly rest for 90 seconds before the task to calibrate recording equipment. EEG signals were acquired from 2 electrodes —one each centered over left and right primary motor cortex. Signals were preprocessed with a 60 Hz notch filter for environmental noise and referenced to the common average. Power envelopes for 1 Hz frequency bands (1–30 Hz) were calculated through Gabor wavelet convolution. Correlations between electrodes were then calculated for each frequency envelope on the first and last 5 runs, thus generating one correlation value per subject, per run. The chosen runs approximately correspond to the first and last week of device usage. These correlations were Fisher Z-transformed for comparison. The first and last 5 run correlations were averaged separately to estimate baseline and final correlation values. A difference was then calculated between these averages to determine correlation change for each frequency. The relationship between beta-band correlation changes (13–30 Hz) and the change in ARAT score was determined by calculating a Pearson correlation. RESULTS/ANTICIPATED RESULTS: Beta-band inter-electrode correlations tended to decrease more in patients achieving greater motor recovery (Pearson's $r = -0.68$, $p = 0.031$). A similar but less dramatic effect was observed with alpha-band (8–12 Hz) correlation changes (Pearson's $r = -0.42$, $p = 0.22$). DISCUSSION/SIGNIFICANCE OF IMPACT: The negative correlation between inter-electrode power envelope correlations in the beta frequency band and motor recovery indicates that activity in the motor cortex on each hemisphere may become more independent during recovery. The role of the unaffected hemisphere in stroke recovery is currently under debate; there is conflicting evidence regarding whether it supports or inhibits the lesioned hemisphere. These findings may support the notion of interhemispheric inhibition, as we observe less in common between activity in the 2 hemispheres in patients successfully achieving recovery. Future neuroimaging studies with greater spatial resolution than available with EEG will shed further light on changes in interhemispheric communication that occur during stroke rehabilitation.

2047

Mental illness public stigma, culture, and acculturation among Vietnamese Americans

Mai Do[1], Jennifer McCleary[2], Diem Nguyen[2] and Keith Winfrey[2]
[1] Tulane University School of Public Health and Tropical Medicine; [2] LA CaTS, Tulane University School of Medicine

OBJECTIVES/SPECIFIC AIMS: Stigma has been recognized as a major impediment to accessing mental health care among Vietnamese and Asian