

Date: 12/19/2017

TITLE: Neuroimaging Biomarkers of Prognosis in Motor Functional Neurological Disorders

NCT # Not applicable

### **3. Study Protocol:**

**3.1a: Overview:** We will use psychometric assessments and structural and functional neuroimaging data collection to study biomarkers of prognosis in motor Functional Neurological Disorders (mFND) patients using a within group design, and secondary between group analyses will also be performed to compare the subgroup of patients with poor prognosis (median split) to controls.

**3.1b: mFND Subjects: Recruitment:** mFND subjects including those with psychogenic nonepileptic seizures (NES), functional movement disorders (FMD) and functional weakness (FW) will be recruited from the Massachusetts General Hospital (MGH) FND clinic. 26 patients (21 women; 5 men) meeting inclusion/exclusion criteria have consented and enrolled. We will include data from these individuals and recruit 49 new patients to bring the total sample size for analyses proposed in this grant to 75. Inclusion criteria: 49 new patients, ages 18-60, meeting clinical diagnostic criteria for mFND will be recruited and longitudinally followed across all aims. 30 mFND subjects will be needed for initial within-group and across-group studies. This cohort will expand to 60 mFND subjects for replication analyses (60 mFND vs. 50+ controls) and to allow sufficient power to rigorously control for confounders (i.e. depression/anxiety, gender differences, medication effects, mFND subtypes). Factoring in a 5-10% exclusion rate due to acquisition issues (head motion) and a 10-15% loss to follow-up in prognosis studies, our overall target enrollment is 75 mFND subjects. Exclusion criteria include any neurological disorder resulting in specific MRI abnormalities (i.e. stroke), epileptic seizures, other movement disorders, poorly controlled medical problems, current alcohol dependence or drug misuse, mania or psychosis, active suicidality and/or head movements at rest.

**3.1c: Control Subjects:** Healthy controls will be recruited by local advertisements.

**3.1d: Baseline Psychometric Data Acquisition:** Eligible mFND subjects participate in a 3-4 hour session to complete self-report measures of functional neurologic symptom severity (motor and sensory symptoms, quality of life (QOL), and dissociation), trauma burden, depression and anxiety. As part of this battery, additional measures assess the spectrum of predisposing, precipitating and perpetuating illness factors, and subjects provide consent for a review of their medical records. A structured clinical interview is also performed by Dr. Perez.

**Symptom Severity Scales:** Subjects complete 4 validated, self-report scales as the primary measures of mFND symptom severity: Screening for Somatoform Symptoms-7: Conversion Disorder Subscale (SOMS:CD): 14-item measure of motor and sensory functional neurological symptoms; Patient Health Questionnaire 15 (PHQ-15): 15-item measure of non-motor functional symptoms including fatigue, gastrointestinal symptoms, and pain; Short Form (36) Health Survey (SF-36): 36-item measure of QOL with 8 sub-domains including physical and emotional health; Somatoform Dissociation Questionnaire (SDQ): 20-item measure of dissociative symptoms.

**Adverse Life Event Scales:** To assess trauma burden, subjects complete 2 self-report scales: Childhood Trauma Questionnaire (CTQ): 25-item measure of childhood abuse and neglect; Life Events Checklist-5 (LEC): 17 item measure of lifetime adverse events.

**Scales for Co-morbid Symptom Characterization:** Subjects complete the Beck Depression Inventory-II (BDI) and Spielberger State-Trait Anxiety Inventory (STAI).

**3.1e: 6 Month Follow-up Psychometric Data Acquisition:** Subjects will complete the above symptom severity and co-morbid symptom characterization scales at 6 months, as well as a questionnaire assessing subjective improvement (5-point Likert scale) and past treatments. To evaluate prognosis, the change in symptom severity scores will be calculated for the 4 symptom severity scales and for affective symptom measures.

**3.1f: Baseline MRI/fMRI/DTI data acquisition:** In a separate session within one-week of the baseline psychometric assessment, subjects participate in a 1-hour MRI scan. T1-weighted MPRAGE, blood-oxygen-level-dependent (BOLD) resting state and diffusion tensor imaging (DTI) scans are acquired on a 3 Tesla Siemens Trio magnetic resonance imaging (MRI) scanner using validated protocols.

## Statistical Analysis Plan

### 4.1 Aim 1 Methods: Identify MRI biomarkers of prognosis in motor Functional Neurological Disorders (mFND) using voxel-based morphometry (VBM) and Cortical Thickness analyses.

**4.2b. Aim 1 Hypothesis Testing: VBM:** MRI scans will be segmented into gray matter, white matter and cerebrospinal fluid components. To control for individual whole-brain volume differences, nonlinear modulation using the Jacobian determinants derived from the normalization process will be implemented. **H1: SPM-based multiple linear regression will test associations between clinical scores of interest (e.g. 6 month change in PHQ-15 ect) and gray matter. Whole-brain corrections for multiple comparisons at the peak voxel-level will use a family-wise error (FWE) rate of  $p < 0.05$ .** Bilateral insula, anterior cingulate cortex (ACC) and amygdala regions-of-interest (ROIs) will be defined in the Pickatlas for small volume corrections (SVC). Since the periaqueductal grey (PAG) is not included in the Pickatlas, a 10mm sphere centered at 1, -29, -12 will be used for SVC based on meta-analysis data. **Cortical Thickness Analyses:** *FreeSurfer* software will correct the T1 acquisitions from each subject for motion and averaged to create a single volume. The averaged volume will be used to reconstruct the gray and white boundaries of the cortical surface. Images will be morphed and registered to an average spherical surface. **H1:** Within-group associations with prognosis will be tested by computing a **general linear model between cortical thickness and variables of interest. A whole-brain cluster-wise correction of 0.05 will be applied to correct for multiple comparisons.** In addition to whole-brain analyses, salience network ROIs will be combined and used as independent variables in regression analyses to allow for the study of cortical thickness associations at the network level with variables of interest (e.g. SOMS:CD, CTQ) as previously reported by the Dickerson lab.

### 5.1. Aim 2 Methods: resting-state functional connectivity (rs-FC) functional MRI (fMRI) markers of prognosis in mFND.

**5.2b. Aim 2 Hypothesis Testing: rs-FC fMRI:** Subjects undergo two 6 minute BOLD rs fMRIs; acquiring 2 scans minimizes loss of data due to head motion and increases signal-to-noise. Acquisition and preprocessing are based on validated methods. Head motion will be accounted for using the latest best practices to correct for potential confounding effects of head motion.

**Seed Based rs-FC:** Hypothesis-driven analyses will determine whole-brain rs-FC topography in relation to a *priori* insula, ACC and amygdala ROIs as defined by the Dickerson laboratory: bilateral subdivisions of the insula (ventral and dorsal anterior, mid, posterior), ACC (dorsal, perigenual, subgenual), and amygdala (dorsal, medial, ventrolateral) will be used to investigate rs-FC in the salience network. To integrate across structural and functional analyses, additional seeds will be chosen from our VBM and cortical thickness findings. **H2:** within group rs-FC associations will be investigated by extracting rs-FC strengths across pre-specified seed regions (Fischer's r-to-z correlation coefficients [ $z(r)$ ]) and correlating these values with covariates of interest (e.g. SOMS:CD, CTQ, change in SOMS:CD). **These connectivity strength values will serve as independent variables in a multiple linear regression analysis with a clinical score of interest entered as the dependent variable. Statistical significance will be set at  $p < 0.05$  correcting for multiple comparisons. Specific network-of-interest analyses will also be performed in relation to the salience network with statistical significance set at an uncorrected p-value of 0.05.** **Graph Theory rs-FC:** Network analyses using graph theory have been developed by Sepulcre. These techniques include stepwise connectivity and weighted-degree analyses. Preprocessing occurs as described for seed-based analyses. Thereafter, the Pearson R correlation coefficient is computed between all pairs of voxels across the whole brain to obtain an association matrix at the individual level. Only positive correlations are considered due to the ambiguous nature of negative correlations in resting-state data. This correlation matrix will then enter specific computational algorithms as described by Sepulcre. **Graph theory protocols will be applied to both within-group and stratified across-group analyses, and a false-discovery rate will be applied for multiple comparisons at  $p < 0.05$ .**

### 6.1. Aim 3 Methods: Identify cingulum bundle and cingulo-insular white matter tract biomarkers of prognosis in mFND using DTI tractography.

**6.2b. Aim 3 Hypothesis Testing: DTI Analyses:** analyses will be based on pipelines within the MGH Center for Morphometric Analysis. For T1 images, cingulate gyrus and cingulo-insular ROIs will be extracted as binary masks using the Destrieux 2010 parcellation. For DTI data, preprocessing includes visual inspection for quality, eddy current correction, skull removal and motion correction using FMRIB Software Library (FSL) software.

Diffusion images will be registered to their own T1 image. Thereafter, FSL probabilistic tractography will be applied using the anterior most portion of the cingulate gyrus as a seed and the posterior most portion as the target. For cingulo-insular tracts, the anterior insular ROI will be the seed and the ACC ROI the target. As a complement to probabilistic tractography, deterministic probability will also be used. White matter integrity measurements will include fractional anisotropy(FA), axial diffusivity(AD), radial diffusivity(RD) and mean diffusivity(MD). **H3**: with-in group associations with prognosis will be tested using multiple linear regression. **A whole-brain cluster-wise correction of 0.05 will be applied to adjust for multiple comparisons across both within-group and stratified between-group analyses.**