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STATISTICAL ANALYSIS PLAN

OFFICIAL TITLE: PET Imaging Study of Amish and Mennonite Patients with *CNTNAP2* Mutations

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Statistical Analysis Plan

The study was designed to enroll 40 individuals (20 affected with the mutation and 20 unaffected) in a cross-sectional biomarker validation study comparing PET mGluR5 binding between *CNTNAP2* mutation carriers and their unaffected first-degree relatives.

Specific Aim 1: Validate PET mGluR5 binding as a downstream marker of mTOR kinase hyperactivity, using individuals with CNTNAP2 as an "experiment of nature" to assess consequences of mTOR kinase-pathway mutations on PET mGluR5 binding.

Primary hypothesis: mGluR5 binding will differ in patients with CNTNAP2 mutations compared with normal controls.

Two-sample t-tests will be used to test for the global effects of CNTNAP2 mutation versus unaffected controls on mGluR5 PET ligand binding, with regional BP_{ND} in DLPFC as the primary outcome variable. Two-sided statistics will be used, to assess potential increases or decreases in mGluR5 PET binding, related to underlying alterations in mGluR5 expression.

Decay-corrected reconstructed PET images will be co-registered to the anatomical MRIs acquired at the beginning of the study, using maximization of mutual information as implemented in SPM8 software. ROIs will be drawn on the MRIs and transferred to the co-registered PET images. Included ROIs will be: HPC and prefrontal (dorso-lateral, medial and orbitofrontal) cortices (19,20), as well as associative, sensorimotor, and ventral subregions of the striatum, thalamus, and occipital, parietal, and temporal cortices. In addition, cerebellum will be included as a reference region to estimate the distribution volume of the non-displaceable compartment (V_{ND}). Time activity curves will be generated from ROI data and subsequently fitted to a 2-tissue compartment model (2TC) with arterial plasma input. Total distribution volume (V_T) will be computed in each region including cerebellum from the estimated rate constants. The binding potential relative to the non-displaceable compartment, BP_{ND}, will be computed indirectly as [V_T (ROI)/ V_T (CER)] – 1.

Specific Aim 2: Evaluate PET mGluR5 binding in other brain regions of potential relevance, including hippocampus (HIPP) and primary visual cortex (occipital pole) in order to determine ideal regions of interest for future intervention studies.

Specific Aim 3: Determine the interrelationship between PET mGluR5 binding across brain regions and symptoms in psychosis disorder patients.

Statistical analysis will be conducted in two phases. The first analysis (i.e., interim analyses, see Power analysis, below) will be conducted following assessment of 10 patients/10 unaffected first-degree relatives, and will provide information to determine whether or not to continue to the second phase of the study. The decision about continuing to phase II will be made by NIMH following review of the phase I data. Of note, the phase I data will be used only for futility analyses to decide on whether or not to proceed with the second study phase.

If the decision is made to proceed to phase II, a second analysis will be conducted using pooled data across the two phases to test the *a priori* hypotheses. Because of the limited sample size and our use of Phase I data solely for the purpose of futility, no statistical correction will be made for the separate analyses conducted following phase I of the study.

Secondary analyses will evaluate group differences in BP_{ND} at additional ROIs including hippocampus and visual cortex. In exploratory analyses, Pearson's correlation coefficient (or

Spearman's rho if data appear non-normal) will be used to investigate relationships between scores on the clinical assessments and BP_{ND} within pre-defined brain regions.

Power Analysis

Interim Futility Analysis Plan

An interim futility analysis will be performed after 10 patients and 10 unaffected first-degree relatives participate in this study. Using the conditional power approach, we have determined the probability that a certain effect size will be found to be significant at the end of the study given the observed effect size at the interim. These probabilities were calculated using simulated data under scenarios matching our design (SAS program available upon request). Assuming a true mean standardized difference (i.e., effect size) of 0.90 between the two groups, if we observe the sample mean standardized difference is less than 0.10 in absolute value between the groups at the midterm (i.e. n=10 in each group), there would be a 23% chance of still finding a significant difference between the groups by the end of study. With an observed midterm difference between 0.10-0.20, 0.20-0.30, or 0.30-0.40 there would be a 30%, 42%, or 50% respectively chance of still detecting a significant difference at the end of study. Thus even with only small differences at the midterm, the chances of still finding a significant result by the end is non-trivial. *Therefore, a cutoff for futility would occur only if the between group effect size is <.10 at the time of mid-point analysis*. Nevertheless, other factors including feasibility and data distribution will be considered and NIMH will have unilateral authority over whether to proceed to phase 2 of the study based upon the results of the interim futility analysis. Screen fails and withdrawals prior to PET scanning will be replaced so the number of evaluable subjects will be reduced only by failure to obtain adequate PET data.

Final Analysis Plan

This sample size is justified by prior *in vivo* human studies that have found significant differences in mGluR5 PET binding with similar sample sizes: 10-23% reductions were detected between depressed patients (n=11) versus healthy (n=11), 5% reductions between sleep deprived versus non-sleep deprived periods in a within person design (n=22). These reductions translate to effect sizes (by scaling the standard errors by sample sizes) of 0.95-1.2 and 0.90. With the proposed sample size of 20 affected individuals versus 20 unaffected 1st degree relatives, with a two-sided alpha level equal to 0.05, we have >80% power to detect effect size differences of 0.90. Assuming the affected versus unaffected differences in mGluR5 PET binding are at least as large as those found due to depression or sleep, we will have >80% power to detect it. For the exploratory aim, we will be able to detect correlations of 0.35 or greater to be statistically significant with alpha = 0.05 and >80% power.