Functional connectivity in presymptomatic juveniles with the gene expansion for Huntington's Disease

David Turaczyk Kolodziej
Mentor: Peggy C. Nopoulos, M.D., Department of Psychiatry

Background and Problem

Huntington's disease (HD) is a progressive, lethal autosomal dominant disorder that manifests itself through behavioral, cognitive, and motor disturbances. It is believed that the pathological hallmark of these symptoms is widespread neurodegeneration that originates in the dorsal striatum of the basal ganglia. HD affects two to eight people per 100,000, and typically begins to present itself, clinically, between 25 and 45 years of age. HD is caused by a trinucleotide repeat mutation (CAG) in the Huntingtin gene (HTT) and can be accurately assessed in subjects at risk for HD (having a parent with HD) by a blood test. Subjects with CAG repeats ≥40 are termed Gene-Expanded (GE) and will develop HD, and subjects with <40 will not develop the disease. Currently, there is no cure available for the disease.

The current etiologic dogma of HD is that the mutant Huntingtin (*mHTT*) results in a gain-of-function toxicity that causes neural damage and degeneration. However, there is also compelling evidence that in addition to this mechanism, loss of function of normal HTT may also be an important mechanism in the disease.[1,2] Normal HTT is necessary for brain development as embryos of HTT monozygous knock-out mice have major abnormalities in CNS development and die shortly after birth.[3] It is also known that HTT is expressed in the brain throughout development [4,5] and plays a vital role in neuronal survival and stability.[6] Therefore, given Huntington's key role in development, a partial loss of function of this protein may manifest in abnormal neural development.

Studies have shown that adult presymptomatic HD subjects exhibit abnormalities in brain structure, cognition, behavior, and motor function long before clinical diagnoses (up to 20 years).[7-13] Although one interpretation is that this is due to early degeneration, another possibility is that *mHTT* leads to abnormalities in growth of brain circuits. These faulty circuits are initially compensated for in childhood and young adulthood but eventually the abnormal circuit succumbs and disease manifests. This theory posits that the partial loss of function of HTT results in a developmental etiology of a neurodegenerative disease. Indeed, studies in the Nopoulos Lab have identified that developmental trajectories in certain brain structures were different between preHD children and controls, suggesting that the pathogenesis of HD begins with abnormal brain development.[14]

HD is initially characterized by its motor element, often referred to as chorea. In fact, the clinical diagnosis of HD is not made until a significant motor abnormality is present. However, behavioral and cognitive deficits can begin to show prior to this point. Thus, subjects who have tested 'gene positive' for HD but have yet to manifest significant motor signs are classified as presymptomatic HD (preHD). To date, most HD therapies have been ineffective. One of the major challenges in these trials is intervening before the degenerative process is in its last stages because, at that point, even the most promising therapies would not be beneficial. Therefore, treatment of HD would be most effective at the presymptomatic stage prior to advancement of the neurodegenerative processes. Additionally, identifying the optimal timing for introduction of treatment therapies would need to be determined. Research focuses on discovering brain structure and function in presymptomatic juveniles may provide insight on the context of HD pathogenesis and appropriate identification of early interventional windows for preHD individuals.[15]

One promising approach to discovering brain structure and function biomarkers for HD pathogenesis prior to onset is by studying functional connectivity networks in presymptomatic HD juveniles. With this method, we can measure altered intrinsic brain connectivity in different resting state

networks between gene expanded juveniles and gene non-expanded juveniles. Past functional connectivity studies have displayed decreased activation of basal ganglia and cortical networks in HD gene carriers.[16,17] Additionally, one study demonstrated significant dynamic functional connectivity differences between HD gene carriers and controls in the default mode network and subcortical domains.[18] These studies suggest abnormal connectivity may be another pathological hallmark of Huntington's disease. Although studies have suggested altered brain connectivity differences in *mHTT* carriers, functional connectivity in resting state brain networks has yet to be studied in preHD juveniles.

As mentioned above, studies have shown there are abnormalities in brain structure of children, adolescents, and adults with preHD up to 20 years before the onset of symptoms. For these reasons, the study of children who are preHD are of high interest to study. Although testing of children at risk for HD below the age of 18 is not allowed for clinical purposes, it can be done for research purposes. This allows for the evaluation of brain structure 30-40 years before onset of HD. If abnormalities are identified at this stage, the etiology is more likely to be considered developmental in nature rather than degenerative.

Aim: To evaluate functional connectivity in the different resting state networks between gene expanded (GE) children (≥40 CAG repeats) compared to gene non-expanded (GNE) children. The GE children are not manifesting symptoms but will develop HD as adults and are therefore preHD. The GNE children will not develop HD and will be used as controls. The study will examine the salience, auditory, basal ganglia, visual, visuospatial, default mode, language, executive, and sensorimotor resting state networks. In addition, we will establish if CAG repeat expansion length has an influence on the degree of altered brain connectivity in each network.

Hypotheses:

1. There will be alterations between GE and GNE groups in functional connectivity of resting state brain networks, specifically the basal ganglia network since the primary effect of *mHTT* seems to be implicated in the development of the dorsal striatum.
2. There will be a dose-effect of CAG repeat such that within each network, greater numbers of CAG repeat will be associated with greater functional connectivity differences from the GNE group.

Role of Student: The recruitment and assessment of the study sample has already occurred. Although some image analysis has already been done on this data set, the functional imaging data has not yet been fully processed, or comparisons done. I will first be trained on the software programs utilized in functional imaging analysis – specifically the CONN toolbox for Matab. This will allow a solid understanding of the functional imaging data and how this program can identify and quantify functional connectivity. I will also be directing the group comparison through this toolbox. Finally, I will be taking a summer course (specific to the Nopoulos lab) on the statistical package, R which will also help in the context of the second aim where network connectivity strength will be correlated with CAG repeat length.

Methods:

Subjects: The Kids-HD study in the Nopoulos lab was an NIH-funded study that recruited and assessed children and adolescents who had a parent or grandparent with HD. The study was conducted between May 2009 and January 2018. This was an accelerated longitudinal design where some subjects had more than one assessment longitudinally (typically 2 years apart). There is a total of 119 GE observations (ages 6-18) and a total of 162 GNE observations (ages 6-18) with good quality MRI scans.

Brain Imaging: Images were acquired on a research-dedicated 3T GE scanner. The following parameters were used for acquisition of the functional images: echo time = 30ms; repetition time = 2,000

ms; flip angle = 80°; slice thickness = 4mm; acquisitionmatrix = 64 × 64. Participants were instructed to keep their eyes closed, stay awake, and not think of anything specific for approximately seven minutes.

Image Analysis: Data will be processed using the CONN toolbox for Matlab. The default preprocessing pipeline within the CONN toolbox will be used, which includes functional realignment and unwarping, slice timing correction, outlier identification, direct segmentation and normalization into stand stereotactic Montral Neurological Institute space,[2] functional smoothing using spatial convolution with a Gaussian kernel of 8mm full width half maximum, denoising with temporal band-pass filtering (0.008 – 0.09 Hz), linear detrending and further reduction of physiological noise using anatomical component-based noise reduction (aCompCor).

Statistical Analysis: Analysis of Covariance (ANCOVA) models will be constructed to compare the average connectivity the defined networks across the two groups (GE vs. GNE). Covariates will include age and sex. For any network that shows significant group differences, we will do a within group analysis (GE only) to evaluate effect of CAG repeat on network connectivity. This will be done using a linear regression model with the CAG as dependent variable, again controlling for age and sex. All analyses will be carried out in RStudio version 1.3.159. Multiple comparisons will be accounted for using the False Discovery Rate (FDR) method.

Summary and Significance

This study will help determine the effects presymptomatic HD has on brain development in various resting state networks by evaluating the functional connectivity differences seen in preHD juveniles based on CAG repeat length. Looking at these relationships provides an opportunity to explore the complexity of HD and identify potential early disease biomarkers in HD.

HD is diagnosed clinically by the manifestation of motor disturbances past an arbitrary threshold. However, there are often abnormalities that occur prior to this diagnosis that are poorly understood. Studying presymptomatic HD children will provide insight into HD in the context of its development. The mutant gene product in HD, Huntingtin, has a key role in brain development. It is possible that, early in life, altered gene products cause minor alterations in the brain development that contribute to the abnormal brain structure characteristic of HD. If HD has a vital portion of its pathophysiology based in abnormal development, then this study could contribute to a conceptual shift in our understanding of this disease (and other degenerative disorders). This, in turn, could lead to better treatment of symptoms and, potentially, a cure.

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Title: Associations between quantitative lung CT markers and inflammatory biomarkers of COPD exacerbation risk.

Student: Anthony El-Sokkary, M1
Mentor: Joseph M. Reinhardt, PhD

Problem/Background Information: Chronic obstructive pulmonary disease is one of the leading causes of morbidity and mortality worldwide (1). Exacerbations of this condition are defined as acute worsening of clinical symptoms that become more frequent as the disease progresses. Severe COPD exacerbations require visits to an emergency room or hospitalization and are responsible for more than 70% of the direct healthcare expenditure related to COPD (2, 3). The risk of symptom worsening is higher in patients with frequent exacerbations (4). Risk stratification for the future exacerbations has been investigated as a means of working towards personalized interventions, particularly with regards to inhaled corticosteroids or phosphodiesterase-4 inhibitors which reduce exacerbations but have their own associated harms (5).

Currently, a prior history of exacerbations is the best-known and most widely used predictor of future COPD exacerbations (4). A history of at least one severe or two moderate exacerbations per year is used to clinically characterize patients at the highest risk of an exacerbation (6). However, prior exacerbation frequency is limited in effectively explaining the degree of risk associated with each episode. Moreover, it fails to provide information about the underlying pathology, thereby mitigating chances of designing patient-specific, personalized interventions.

Automatically extracted biomarkers from chest CT scans at total lung capacity (TLC) and residual volume (RV) are increasingly used to subphenotype COPD. Recently, research performed here at the University of Iowa has demonstrated that imaging biomarkers extracted from scans from the Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS) cohort demonstrated strong predictive values for exacerbation over the course of 5 years. Specifically, CT density gradients (CTDG) and increased CT density (ICDT) patterns combined with other airway measures yielded an AUC of 0.875 (95% CI 0.872,0.878). This study hypothesized that CTDG could be driven by inflammation around the bronchovascular bundles/fissures and emphasized that its results focused on predictive estimates and not causality. Thus, there is value to be derived from correlating these results of CT-base biomarkers of COPD exacerbation risk with serum-based markers of inflammation.

Soluble receptor for advanced glycation end products (sRAGE) is one such biomarker whose serum reduction has been associated with more severe GOLD (Global Initiative for Chronic Obstructive Lung Disease) stage, exacerbation history, and forced expiratory volume (FEV) % of predicted, and more extensive emphysema (7, 8, 9). Additionally, panels of inflammatory

biomarkers CRP, Fibrinogen, and Leukocyte count have demonstrated increased risk of frequent exacerbations (10). As these markers are already documented within the SPIRMOMICS data, correlations between sRAGE and simultaneous changes in CRP, Fibrinogen, and Leukocyte count may provide further evidence of an inflammatory component explaining the relationships of CT-based biomarkers described above.

Hypothesis: There is an association between CT textures indicative of COPD exacerbation risk and reduced sRAGE levels. Additionally, simultaneous increases in CRP, Fibrinogen, and leukocyte count is associated with CT textures indicative of COPD.

Aims:

1. Determine if there is an association between reduced sRAGE levels and CT textures associated with increased COPD exacerbation.

2. Determine if there is an association between CT textures predictive of COPD exacerbation risk and simultaneous elevations of CRP, Fibrinogen, and Leukocyte count

3. Assess if other variables of interest may be associated with extracted CDGT and ICTD patterns.

Methods: CT scans from the SPIROMICS cohort data will be analyzed for pulmonary biomechanical markers of deformation, airway measurements, parametric response mapping, and adaptive multiple features method-based markers (AMFM). AMFM data will display as a percentage of the total lung volume in the scan that meets trained algorithms for patterning similar to glass-like opacity textures, emphysema-like, ground glass-like reticular interstitial patterns, bronchovasculature, honeycombing-like textures, and non-diseased normal-like tissue. Data from the SPIROMICS cohort will not include any identifiable information. The data will also be screened for subjects that both had CT scans performed and who provided blood samples for biomarkers of inflammation during their baseline visits.

Role of the Student: My role will be to gather CT scans from the SPIROMICS database, run them through quantitative analyses, and run statistical analyses of the data from investigate associations between biomarkers collected by the study (sRAGE, CRP, Fibrinogen, and Leukocyte count, etc.) and processed CT texture pattern data.

Significance of Research:

By determining if associations exist between predictive CT-based biomarkers and inflammatory serum markers, this project would provide further evidence for inflammation being the primary mechanisms behind COPD pathogenesis and specifically with regards to risk for future exacerbations. Understanding the pathogenesis and phenotypes of COPD exacerbation risk is

important for discovering new targets for interventions and creating personalized treatment plans based on patient risk stratification.

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Metabolomic Profiling of Human Vestibular Schwannoma and Meningioma Before and After Radiation Therapy

Student: Hashim Syed

Mentor: Dr. Marlan Hansen, MD

Problem

Vestibular schwannomas (VS) and meningiomas account for approximately 10% and 37% of all primary intracranial neoplasms, respectively, with meningiomas being the single most common primary intracranial tumor [1]. They share multiple key genetic and anatomic features, which makes studying them in parallel advantageous, with the most important shared feature being frequent mutations in the *NF2* tumor suppressor gene. In most cases that require treatment, these tumors can be treated with surgical resection and/or radiation with good outcomes. However, when those treatments fail no further options exist.

Metabolomic profiling is a method in which the relative quantities of metabolites present in tissue samples can be demonstrated [2-4]. Such techniques have been utilized in multiple tissue types and pathologic processes to date, including cancers such as breast, lung, and hepatocellular cancers, and evaluating for graft rejection following organ transplantation [5-8]. However, few efforts have been made to date to characterize the metabolomic profile of low-grade intracranial neoplasms such as VS and meningiomas [2,9,10]. Doing so could establish new techniques for prognostication and predictive analytics, which could then be used as an additional consideration during therapeutic decision-making. The data will also likely point to novel or combined therapeutic strategies.

Hypothesis

We hypothesize that 1) radiation induces specific metabolomic changes in meningiomas and schwannomas, and 2) these changes arise from alterations in targetable metabolic pathways. Results from these studies will facilitate our long-term goal to identify key metabolic pathways in meningiomas and schwannomas that can be therapeutically targeted to limit tumor growth and increase the efficacy of radiotherapy. Doing so could dramatically improve both the duration and quality of life in some of our most difficult patients.

Background Information

While metabolomic analyses have been performed in a variety of tissue types, only one publication to date has discussed this in meningiomas, and none in schwannomas [2]. In addition, no prior metabolomic analysis has been performed pre- and post-radiation for these tumors. We will therefore begin by performing non-targeted metabolomic analyses of primarily resected human VS and meningioma tissue. This tissue will provide the baseline metabolomic information from which further analyses will be based. Such tissue offers the most accurate representation of the metabolic milieu of the natural tumor environment. However, as this primary tissue is flash-frozen and key metabolic changes begin to occur almost instantly after resection, it is not a suitable tissue to test the effects of therapies administered post-resection.

Hence, in parallel we will use additional tumor tissue to establish primary cell cultures and orthotopic xenografts in mice. Having actively growing tumor tissue in vitro allows us to test the effects of ionizing radiation on the tissue in a dose-escalating fashion. These cultures are likely to vary from in vivo tumors

in many ways, but they will allow us to efficiently develop testing protocols for subsequent testing in our murine xenograft models. This is because, after radiation, the metabolic networks are likely to change on a time scale of minutes to hours, so finding the ideal timing for metabolomic analysis after the tumor cells are radiated will be critical to our results. We will grow tumors as orthotopic xenografts in mice, allowing us to test metabolomic changes in a living model that will mimic the natural tumor microenvironment in ways that in vitro models cannot.

Finally, we will measure response patterns of increasing doses of radiation (0-40 Gy) on primary human VS and meningioma cultures and xenograft. While radiation rarely eliminates these tumors, they often become quiescent. The metabolic changes induced by radiation will provide insights into potential targets to enhance radiosensitivity; they also will identify metabolic profiles associated with tumor quiescence.

Thus far, we have established the feasibility of this project by establishing primary cell cultures from human meningioma samples obtained in December 2020, exposing subgroups of these cultures to gamma radiation in a dose-escalating fashion, and subsequently using non-targeted metabolomics to measure relative concentrations of 75 metabolites. We found significant changes ($p<0.05$) in eight metabolites after 10 or 20 Gy radiation, and near-significance ($p<0.10$) in four metabolites.

In addition to cultured cells exposed to varying doses of radiation, we have also successfully obtained metabolomics data for 6 flash-frozen meningioma tumor samples. Although we have not yet evaluated enough samples to discern key pathway alterations present in meningiomas, this provides a key proof of feasibility for analyzing previously banked flash-frozen tumor samples. Notably, the Hansen lab has over 200 flash frozen human VS samples which could be analyzed for the purposes of this proposal.

Methods and Student Role

We will perform gas chromatography-mass spectroscopy (GC-MS) analysis on prepared tissue samples flash-frozen in liquid nitrogen at the time of surgical resection and on mouse xenograft specimens. Sample analysis will be performed on the Thermo Q Exactive GC-MS—which profiles >100 standard metabolites—and specialized software (MetaboAnalyst, Montreal, Quebec, Canada) will then be used to determine relative metabolite quantities [11,12]. Samples will be run in batches; between the collection time and batch analysis the samples will be stored in an enclosed container at -80C.

For in vitro testing, primary human VS and meningioma cultures from freshly resected tumors will be established as previously described [16,17]. We will then divide the culture samples into multiple dishes and deliver ionizing radiation via 15-minute exposure to cesium, which emits gamma rays and is reflective of the ionizing radiation used in clinical practice or perform a 15-minute sham treatment. We will begin dosing protocol with four dishes, with zero (sham), 3 Gy, 10 Gy, and 20 Gy initial doses. Twenty-four hours after radiation, the cells will be delivered to the University of Iowa Metabolomics Core facility to initiate GC-MS analysis. We will repeat the experiment on a minimum of 10 cultures derived from separate primary tumors. We will then use the experimental data derived from this testing to select the ideal radiation dosages to be delivered to the xenografts.

For in vivo testing, intradural, extra-axial human VS and meningioma xenografts derived from acutely resected tumors will be established in nude mice as previously described [18-20]. For each tumor sample, at least 4 mice will be injected. MRI will be used to confirm tumor implantation. After a minimum of 30 days, and after confirming sufficient tumor mass to allow for metabolomic analysis, half of the tumors will be subjected to ionizing gamma radiation from a cesium source. The other half will receive sham radiation, wherein they will be prepared for the radiation treatment in the same fashion, but no radiation will be given. Next, 24 hours after the treatment, the mice will be anesthetized, and the implanted tumor will be biopsied. Following adequate tumor sampling, they will be euthanized. It is essential to perform sampling for metabolomic analysis prior to euthanasia, as tissue ischemia immediately alters relative

metabolite frequencies. To ensure accurate tissue sampling, frozen sections of the tumor will be cut from remaining tissue after sufficient sample has been sent for metabolomic analysis. For cell type identification, we will use antibodies against EMA to label meningioma cells and S100 for VS cells [21-24]. Nuclei will be stained with DAPI.

In addition to assisting with the above outlined methodology, and after receiving training from Drs. Hansen and Dougherty, I will specifically help conduct mass spectroscopy analysis, establish xenografts in mice, check on mice after surgery, assist with radiation, tumor sampling, fluorescent microscopy, cell counting, and metabolomic and pathway analysis.

Significance of Research

Vestibular schwannomas (VS) and meningiomas account for approximately 10% and 37% of all primary intracranial neoplasms, respectively, with meningiomas being the single most common primary intracranial tumor [1]. Surgical resection and radiation are both considered first-line treatments, but when those fail patients are left with no further options [13,14]. Uncontrolled tumor growth can result in devastating neurological consequences [15]. Hence, novel therapeutics are critically needed to fill this treatment gap, particularly for patients with Neurofibromatosis type 2 who develop multiple meningiomas and schwannomas and ultimately succumb to advanced tumor burden.

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