Foundation for PSP I CBD and Related Brain Diseases

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2010 INTERNATIONAL RESEARCH SYMPOSIUM

Embassy Suites Hotel – San Diego, CA

Thursday, November 18, 2010

ABSTRACTS

Structural Polymorphism of Tau Filaments in CBD

Martin Margittai, PhD, University of Denver

Lay Abstract:

Corticobasal Degeneration (CBD) and Alzheimer's disease (AD) are fatal neurodegenerative diseases that are characterized by large aggregates composed of the protein tau. When magnified under the electron microscope these aggregates have a filamentous appearance. Similar aggregates are found in >20 other neurodegenerative diseases commonly referred to as tauopathies. The shapes of tau filaments vary between different diseases and may include paired helical-, straight-, or ribbon-like filaments. Whether such variations in shape translate to differences on the molecular level is currently unknown. The adult human brain contains six different types of tau molecules that vary in their amino acid sequence. While aggregates in AD carry all types of tau molecules, aggregates in CBD contain only a subset thereof. How these differences contribute to structural diversity is not known. The objective of this study was to address these gaps in our understanding by using seeds as templates for filament growth and sensitive reporter technologies for structure determination. We hypothesized that a set of different filament structures exists that could explain some of the phenotypic diversity observed between different tauopathies. Our project entailed two specific aims: 1) Development of a novel molecular seeding assay, and 2) Determination of structural polymorphism of tau filaments. In a first step towards these goals we identified a common core structure in filaments formed from different molecules of tau. We determined that these molecules coassemble to form mixed filaments suggesting that individual filaments in AD are distinct from those in CBD. In a second step we established an *in vitro* seeding assay that propagated the structure of the seeds as monitored by sensitive reporter techniques. This assay was then applied to propagate filaments isolated from CBD and AD tissue. We identified subtle structural differences. Future experiments will expand these studies and investigate the potential impacts on filament spreading.

Scientific Abstract:

Corticobasal Degeneration (CBD) and Alzheimer's disease (AD) are fatal neurodegenerative diseases that are characterized by filamentous inclusions of the microtubule associated protein tau. Similar inclusions are found in >20 other neurodegenerative diseases commonly referred to as tauopathies. The morphology of tau filaments varies between different diseases and may include paired helical-, straight-, or ribbon-type filaments. Whether the differences in morphology translate to the molecular level is currently unknown. The adult human brain contains six isoforms of tau, which have either 3- or 4- microtubule binding repeats. While filamentous inclusions in AD carry all isoforms, inclusions in CBD contain only the 4-repeat forms. How these differences contribute to conformational diversity is not known. The objective of this study was to apply conformational templating in conjunction with fluorescence and electron paramagnetic resonance spectroscopy to determine tau structure at the level of the backbone fold. We hypothesized that a set of different filament conformations exists that could explain some of the phenotypic diversity observed between different tauopathies. Our project entailed two specific aims: 1) Development of a novel molecular seeding assay, and 2) Determination of structural polymorphism of tau filaments. In a first step towards these goals we identified a common core structure of parallel, in-register arranged □-strands in filaments of 3- and 4- repeat tau. We determined that these isoforms coassemble to form mixed filaments suggesting that individual filaments in AD are distinct from those in CBD. In a second step we established an *in vitro* seeding assay that propagated the conformation of the seeds as monitored by sensitive reporter techniques. This assay was then applied to propagate the conformation of filaments isolated from CBD and

AD tissue. We identified subtle structural differences. Future experiments will expand theses studies and investigate the potential impacts on filament spreading.

Regulation of Tau Pathology by the Microglial Fractalkine Receptor
Kiran Bhaskar, PhD, Cleveland Clinic

Lay Abstract:
Tauopathies, including AD, are increasingly common neurodegenerative diseases in our aging population with characteristic clinical signs including changes in personality, social behavior, and language ability, difficulties in thinking and making decisions, poor coordination and balance, psychiatric symptoms and dementia. Currently there are over 5 million people in the US with AD, while millions more are affected by non-AD tauopathies. Analysis of the brain pathology suggests that these tauopathies are characterized by aggregation of a protein called tau as “tangles” within the nerve cells; hence, the name “tauopathies.” While several research studies have suggested accumulation of tangles within nerve cells is the basis for neuronal loss in tauopathies, the exact cause for the development of tangle pathology is unknown.
Inflammation, induced by immune cells of the brain called microglia, was traditionally considered to be a beneficial but trivial secondary response in tauopathies, where it can functionally eliminate cellular debris. However, cellular studies indicate that, upon activation, microglia actually become neurotoxic by producing variety of molecules that can kill nerve cells. Recent evidence suggests an association between microglial activation and tau aggregation in human cases and mouse models of tauopathies. However, it is unclear how microglia specifically promotes tangle pathology and/or dementia within the brain. To test this hypothesis, we utilized genetically engineered mice that model tauopathies (hTau mice), which overproduce tau protein within the brain and display tangle pathology as well as dementia, modeling human tauopathy. In addition, we applied information learned from our previous studies that removal of a protein called the fractalkine receptor from microglial cells not only disrupts their ability to signal to nerve cells, but also induces excessive microglial activation in response to inflammatory or neurotoxic stimuli, resulting in enhanced neurotoxicity.
First, we observed that hTau mice show progressive microglial activation that coincides with tangle pathology, similar to what occurs in humans. Second, we challenged mice (non-transgenic, hTau and fractalkine receptor-deficient mice) with inflammatory stimuli and found that inflammatory stimuli enhanced tangle pathology in non-transgenic and hTau mice, which was further enhanced in fractalkine-receptor deficient mice. Finally, when the fractalkine-receptor was removed in hTau mice, it accelerated tangle pathology and worsened cognitive function. These results suggest that chemically or genetically enhancing microglial-specific inflammation accelerates tangle pathology and memory impairment in transgenic mice.
Next, to determine how microglial inflammation causes tangle pathology in neurons, we performed cell-culture experiments. In a two-compartment cell-culture dish, we cultured microglia in one compartment and neurons in the other. After 24 hours of culturing together, we measured tangle pathology in neurons and examined the media for tangle-inducing soluble factors released from microglial cells. Our results suggest that microglial-derived soluble factor(s) such as interleukin-1 (IL1), a pro-inflammatory cytokine, can act on neurons and activate a stress-activated protein called p38 mitogen-activated protein kinase (p38 MAPK), which in turn can cause tangle pathology within neurons. Blocking either IL1 or p38 MAPK via respective pharmacological inhibitory compounds rescues neuronal tangle pathology from microglial effects. We have also confirmed this in a genetically engineered mice that lack a receptor for IL1 and are resistant from developing tangle pathology following inflammatory stimuli. Together suggesting that microglia-specific brain inflammation induces tangle pathology via IL1-p38 MAPK signaling and that future therapy targeting fractalkine-IL1-p38 MAPK may be useful in blocking tangle pathology in patients with AD and related tauopathies.

Scientific Abstract:
Aggregates of the hyperphosphorylated microtubule associated protein tau (MAPT) are an invariant neuropathological feature of Alzheimer’s Disease (AD) and related tauopathies. While increasing evidence suggests that alterations in microglial activation is a common feature of tauopathies, the exact pathogenic role of microglia in disease pathogenesis remains unclear. Here we show that neuroinflammation mediated by toll-like receptor (TLR4) or genetic deletion of the microglial fractalkine receptor, CX3CR1,

promotes MAPT phosphorylation and aggregation through a mechanism that is mediated via the p38 mitogen activated protein kinase (MAPK) signaling pathway. First, we demonstrate that lipopolysaccharide (LPS)-mediated microglial activation induces hyperphosphorylation of endogenous mouse MAPT in non-transgenic mice that is further enhanced in mice lacking CX3CR1 and is dependent upon functional toll-like receptor 4 and interleukin 1 receptors. Second, the humanized MAPT transgenic (hTau) mouse model of tauopathies lacking CX3CR1 exhibits enhanced MAPT hyperphosphorylation and aggregation, formation of silver positive neurofibrillary tangles as well as behavioral impairments that correlated with increased levels of active p38 MAPK and its downstream target, activation transcription factor 2 (ATF2). Finally, in vitro experiments utilizing primary microglia and cortical neurons demonstrate that microglial activation elevates the level of active p38 MAPK and enhances MAPT hyperphosphorylation within neurons that can be blocked by administration of an interleukin 1 receptor antagonist and a specific p38 MAPK inhibitor. Taken together, our results demonstrate that fractalkine-receptor deficiency in microglia directly induces MAPT pathology via activation of TLR4/IL1/p38 MAPK pathway and suggests that CX3CR1 and/or p38 MAPK signaling pathways may provide novel therapeutic targets for human tauopathies.

Characterization of Tauopathy in Zebrafish
Edward Burton, MD, DPhil, University of Pittsburgh

Lay abstract:
One of the problems thought to be important in the development of PSP is the accumulation of abnormal clumps of proteins, called neurofibrillary tangles (NFTs), within nerve cells in the brain. NFTs are made from a protein called Tau. Availability of a model in which Tau formed NFTs similar to those found in PSP might enable us to isolate chemicals that prevent NFTs from forming in the brain, or from adversely affecting nerve cells. These chemicals could form the starting point for designing drugs to treat PSP. The zebrafish is a small black-and-white striped fish; surprisingly, the fish brain is organized in a similar way to the human brain in a number of ways that are relevant to studying PSP. We have generated a zebrafish model that reproduces many features of PSP in the zebrafish brain, by making genetically-engineered zebrafish that either make too much Tau, or make an abnormal type of Tau, in their brain cells. Our studies show that genetically-engineered Tau zebrafish show behavioral abnormalities and do not move properly. Tau zebrafish also develop progressive loss of brain matter and clumps of Tau that resemble NFTs. Our results indicate that the zebrafish is a valid model in which to study PSP. We are now modifying this model into a format suitable for screening many thousands of chemicals in order to find ones that prevent the animals from developing PSP-like problems. Importantly, chemicals isolated by using this model could form the basis for novel drugs to treat PSP.

Scientific Abstract:
Our work is currently focused on generating genetically-modified zebrafish that recapitulate biochemical and cellular abnormalities of PSP. Our goal is to use these models in high-content assays for drug discovery. The first stage of the work was to establish proof of concept that the zebrafish could be used to model Tauopathy. We isolated a cis-element derived from the zebrafish eno2 gene in order to construct transgenic zebrafish expressing human Tau. A 12kb eno2 element was active in driving transgene expression in neurons throughout the brain and spine from 48 hours post-fertilization through adulthood. We have generated multiple lines of Tg(eno2:MAPT) animals expressing human 4R Tau or mutants associated with disease. Transgenic animals are viable and survive to adulthood, but show age- and expression level-dependent changes in behavior and abnormalities of reproduction. These phenotypic abnormalities are accompanied by neuropathological and biochemical abnormalities. Age-dependent reduction in brain mass with respect to control animals is accompanied by evidence of microgliosis and microglial activation, and the formation of neuronal cytoplasmic accumulations of Tau. Similar to PSP, 4R Tau in the zebrafish model is hyperphosphorylated and shows abnormal solubility. Some of the inclusions show argyrophilia, suggesting the formation of neurofibrillary tangles. These data confirm that the model replicates key pathological changes of Tauopathy in a progressive age-dependent manner, validating the zebrafish model for the study of PSP. Our current work is focused on developing a model that develops a more severe phenotype at larval stages, allowing high-throughput small molecule screens using automated motor assays to discover novel chemical modifiers of the Tauopathy phenotype. We have constructed

conditionally expressing lines and have optimized assays of motor and oculomotor function to allow for screening approaches using these models.

Characterization of a Mutant Tau Transgenic Mouse Model as a Drug Discovery Tool
Hanno Roder, PhD, TauTaTis, Inc.

Lay Abstract:
TTT-3002 is a drug designed to inhibit abnormal processes affecting tau protein, which cause nerve cell degeneration in a variety of progressive neurological disorders, such as Alzheimer's disease, Progressive Supranuclear Palsy, Corticobasal Degeneration and others.
As a prerequisite to long-term exposure of transgenic mice to TTT-3002, which harbor disease-causing mutations of the tau gene, we first successfully verified tolerability, absence of adverse effects, in particular of normal brain function, and the ability to maintain sufficient drug levels in brain over extended periods of time of regular oral dosing.
We started treatment of transgenic mice at an age where some animals were expected to be at an early stage of disease, while a majority should have been at an already very advanced stage. After three months of treatment we found that in those mice deemed to have been at an early stage at initiation of treatment did not appear to have experienced any loss of brain mass during treatment. These mice also had less, and in some cases even almost no disease-specific changes to tau protein relative to untreated mice. They also showed less impairment in memory tests.
On the other hand, the group of mice that was deemed to have been at a very advanced disease stage at the start of treatment did not show any benefit. However, a deeper analysis of the disease process suggests that abnormal changes to tau in these mice had escalated into a type that is resistant to TTT-3002, but is an artifact of the mouse model, and cannot be expected to occur in humans. Therefore, the lack of treatment response in mice of advanced disease stage cannot be interpreted as predicting a similar outcome in human patients of advanced stage. In any case, however, a therapeutic effect of TTT-3002 in slowing or halting at least the early stage disease process in humans can now be expected.

Scientific Abstract:
MTD Studies: In pilot studies with non-transgenic littermates of the P301L-mutant tau transgenic mouse model rTg4510 the maximal tolerated dose (MTD) for twice daily application of the hydrochloride salt of TTT-3002 in a solid form was determined to be 10 mg/kg for females, and 2.5 mg/kg for males, producing comparable plasma and brain exposures, however. The dose limiting effect was a rapidly reversible dyspepsia. Brain levels at MTD were sustained at the targeted level of 300-400 nM without much variation.
Baseline Studies: Non-transgenic littermates dosed twice daily at MTD for a period of three weeks showed equal performance in the Morris Water Maze (MWM) test of learning and memory compared to vehicle controls. Normal tau phosphorylation and activation status of JNKs were unaffected by treatment.
Because of a previously identified significant sex dependence of onset and severity of tau pathology in this model we used only female rTg4510 mice with the more aggressive neurodegenerative phenotype. Dosing was initiated at 5 months of age, where previous analysis had indicated a bimodal presentation of pathology, whereby in females a fraction of about 1/3 would produce only very low levels of extractable PHF-tau (≤1% of transgenic tau converted to pathological tau) and low histological tau burden with large areas still spared from pathology, while a fraction of about 2/3 would have ≥10% PHF-tau and tau pathology affecting essentially the entire brain.
Study Schedule: MWM performance was assessed (i) before, (ii) after 2 weeks, and (iii) after 3 months of dosing, whereafter brains were resected and cortical weights were recorded in a blinded fashion. One half of each brain was homogenized and the other was fixed in formalin. In prior studies cortical weight was identified as the potentially most sensitive, and also most relevant, indicator of anti-neurodegenerative drug activity, followed by PHF-tau levels, and behavior
Effect on Cortical Atrophy: A fraction of 1/3 of the treated mice had cortical weights that did not occur in controls and corresponded to weights expected in 5 month old female rTg4510 mice, i.e. the age of onset of treatment, implying a complete arrest of neurodegeneration in that group (responders). No treatment effect was evident in cortical weights of the remainder of the treated cohort (Non-responders).

Effect on PHF-tau Levels: Although PHF-tau levels were generally not well correlated with brain atrophy in controls, the responder group had significantly lower levels of PHF-tau relative to the vehicle cohort, and in a few cases nearly undetectable levels, never seen in controls in females of that age (8 months). Non-responders did not show significantly reduced PHF-tau levels. More detailed phospho-epitope analysis of PHF-tau produced evidence for a two-stage process of tau pathology in this model.
We also obtained evidence for inhibition by TTT-3002 of a pathological form of MAP-kinase activation predicted by our cell models in the treated cohort.
Effect on Learning/Memory: Prior experience of generally poor performance of rTg4510 mice in the MWM paradigm was confirmed. Nonetheless, less impairment of the responder group relative to the vehicle group could be demonstrated.
Mechanistic analysis revealed evidence that tau pathology in the rTg4510 mouse model proceeds in two distinct stages representing fundamentally distinct mechanisms of PHF-tau formation, whereby only the first stage is responsive to TTT-3002, while the second stage is not. This second stage appears to be tied to specific issues of mouse brain development, which do not occur in other mammals, including humans. The absence of therapeutic effects in non-responder mice is therefore not relevant for human pathology. It also expected that much more dramatic treatment results could be obtained if treatment was initiated at an age where all mice would be in the first stage of pathology.

A Pilot Clinical Trial of NAP(AL-108) for CBD and FTLD with Predicted CBD Pathology
Adam Boxer, MD, PhD, University of California, San Francisco

Abstract:
Background: There are no effective treatments for FTLD. Approximately half of all FTLD cases have underlying tau-related pathology. Clinical FTLD syndromes most strongly associated with tau pathology include Progressive Supranuclear Palsy (PSP), Corticobasal Syndrome (CBS) and Progressive Nonfluent Aphasia (PNFA). Davunetide (NAP) is a drug derived from Activity Dependent Neurotrophic Protein that has beneficial effects on memory and tau phosphorylation in tau mutation transgenic mice, and has demonstrated a symptomatic benefit on attention and working memory in humans with amnestic MCI. The purpose of the study was to collect pilot data on davunetide effects in individuals with predicted tauopathies.
Methods: We conducted a 12 week, randomized (2 drug : 1 placebo), double-blind, placebo-controlled trial of davunetide 15 mg delivered twice daily intranasally in 12 subjects with clinical FTLD syndromes with predicted underlying tau pathology. The last patient, last visit was planned to occur in August, 2010. The primary outcome measure was safety and tolerability as measured by treatment emergent adverse events and clinical laboratory and ECG data at 12 weeks vs. baseline. Secondary outcomes include 12 week vs. baseline changes in: the PSP Rating Scale, the UPDRS, the Clinician's Global Impression of Change, the Schwaab and England ADL scale, the CDR-FTLD, the RBANS cognitive battery, the EXAMINER executive function battery, the NPI, horizontal and vertical saccade function (latency, gain, velocity measured using an infrared eye tracker), volumetric and connectivity measurements on MRI scans, and CSF tau, ptau and Abeta levels.
Results: At baseline, 6 PSP, 3 PNFA and 3 CBS subjects were enrolled. Baseline demographics were: gender (4M/8F), age 64.8±5.0, CDR-SB 4.1±2.1, MMSE 26.4±1.8, PSPRS 35.8±12.6, UPDRS 33.9±15.8 and SEADL 66.6±20. Preliminary safety and efficacy data will be reported.
Conclusions: This pilot study will provide useful data to help conduct a pivotal, phase 2/3 clinical trial of davunetide in PSP.
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GRAPE-Derived Polyphenols as Potential Therapeutic Agent in Tauopathies
Giulio Pasinetti, MD, PhD, Mt. Sinai School of Medicine

Lay abstract:
Abnormal folding of the microtubule-associated protein, tau, leads to the generation of intracellular paired helical filaments (PHFs). PHFs are a key neuropathologic feature in multiple neurodegenerative disorders, including progressive supranuclear palsy (PSP), which are collectively referred to as "tauopathies".

Current hypotheses suggest misfolded tau induces a “toxic gain of function” that contributes to the onset and progression of neurodegeneration. We recently identified a grape-seed polyphenolic extract (GSE) capable of interfering with tau misfolding and destabilizing misfolded tau paired helical filaments that are formed. We hypothesize the GSE may attenuate misfolding of tau protein that leads to the generation of filamentous tau aggregates in tauopathies, and thereby reduce tau-mediated pathological phenotypes in PSP and other tauopathies. In feasibility studies we demonstrated GSE suppresses neurodegeneration in a fruit fly model of tauopathy. We continued to explore the role of GSE in a mouse model of tauopathy mouse (referred to as the JNPL3 the mouse model) that is characterized by development of tau neuropathology, motor impairment and early mortality. We found GSE treatment significantly reduced tau neuropathology, and coincidentally improved motor functions and reduced mortality in JNPL3 mice. In parallel studies, we demonstrated that GSE is also effective in reducing tau neuropathology in a second mouse model of tauopathy (referred as the TMHT mouse model which develops tau neuropathology and cognitive impairment). Moreover, our evidence suggests that GSE may reduce tau neuropathology in the brain, in part, by modulating specific molecular signaling processes. While misfolded tau in the cell interferes with normal cell functions and induces neurodegeneration, recent evidence suggests misfolded tau (e.g., PHF) released by degenerating neurons into extracellualar space (space outside of and surrounding cells) may provoke tau misfolding in neighboring “naive” cells, and thereby help induce spreading of tau pathology in the brain. We used isolated PHF from postmortem Alzheimer’s disease brain specimens (AD-PHF) to explore the mechanisms by which extracellular misfolded tau may induce abnormal tau aggregation inside the cell, and to assess whether GSE may interfere with tau spreading mechanisms. In preliminary studies, we found pretreatments of cultured neuronal cells with the GSE significantly reduced the efficacy of AD-PHF to induce tau misfolding inside the cell. Collectively, our evidence suggests GSE may protect against tau-mediated pathogenesis by modulating multiple mechanisms, and provide the basis for developing GSE for the prevention and/or treatment of PSP and other tauopathies.

Noninvasive Cortical Stimulation for Motor and Non-Motor Features of PSP and CBD

Choi Deblieck, PhD & Allan Wu, MD, University of California, Los Angeles

Abstract:

We investigated the effects of repetitive transcranial magnetic stimulation (rTMS) on corticomotor excitability (CE), motor behavior, and mood in patients with progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD).

We hypothesized that rTMS over primary motor cortex (M1-rTMS) would affect CE and motor behavior while rTMS over dorsolateral prefrontal cortex (DLPFC-rTMS) would affect mood. Patients were randomized to receive M1-rTMS, DLPFC-rTMS, or sham-rTMS at 90% motor threshold in 3 sessions with assessments compared before vs. after stimulation. 4 CBD patients received 1Hz-rTMS (for dystonia) and 6 PSP patients received 5Hz-rTMS (for akinesia-rigidity) over affected M1. All patients received 5Hz-rTMS over left DLPFC.

CE was assessed with motor evoked potentials (MEP) and cortical silent periods (CSP), motor behavior with maximal and comfortable (COM) tapping speed, mood with a visual analog scale. Across all patients, only M1-rTMS significantly reduced sadness (p=0.03).

M1-rTMS and DLPFC-rTMS in PSP tended to improve COM on the less affected hand (M1=-185.4ms/DLPFC=-109.7/sham=-28.5) whereas it tended to slow it down in CBD (M1=196/DLPF=74.5/sham=33.6). In both cases, M1-rTMS showed a slightly greater effect size compared to DLPFC (M1=0.12/DLPFC=-0.05/sham=-0.08).

At rest, M1-rTMS tended to increase MEP amplitude in the affected hand in PSP while it tended to reduce MEP amplitude in CBD (PSP=2.8mV/CBD=-3.4). Conversely, under active conditions, M1-rTMS in PSP tended to reduce MEP amplitudes with no effect on CSP while it tended to increase MEP amplitude and lengthen CSP in CBD (PSP=-1.3mV/CBD=1.1). In both groups, DLPFC-rTMS showed no significant changes in CE.

These preliminary results suggest that effects of M1-rTMS in PSP and CBD may produce weak effects on CE, tapping, and sadness ratings relative to DLPFC/sham-rTMS and future studies of M1-rTMS may be warranted to correlate with patient symptoms.

Pedunculopontine Deep-Brain Stimulation in PSP and CBD
Elena Moro, MD, PhD, University of Toronto

Lay Abstract:
Deep brain stimulation (DBS) involves delivering electricity to specific brain regions through surgically implanted electrodes. There is recent evidence suggesting that DBS in a new brain surgical target, the pedunculopontine nucleus (PPN), may improve walking and postural problems in patients with Parkinson's disease. As walking and balance are major struggle in progressive supranuclear palsy (PSP), we have designed a study aimed at investigating safety and efficacy of PPN DBS in patients with PSP. Six PSP patients will have unilateral PPN DBS surgery. Motor and non-motor symptoms will be evaluated before and after surgery up to 1 year. A specific study of walking and balance will also be conducted to assess potential improvement from PPN DBS. Beneficial effects obtained with PPN DBS in the treatment of walking and balance disturbances in PSP will likely improve the quality of life in these patients.

Scientific Abstract:
Background: Gait impairment and postural instability are disabling motor features of progressive supranuclear palsy (PSP). In Parkinson's disease (PD), deep brain stimulation (DBS) of the pedunculopontine nucleus (PPN) is currently investigated as a new surgical therapy to address axial signs (gait and balance).
Objectives and hypotheses: To investigate whether unilateral PPN-DBS is safe and effective in PSP patients. We hypothesize that PPN-DBS will improve gait, postural instability and quality of life, without worsening other motor signs and cognition. PPN-DBS will possibly increase activation of the frontal cortex during PET scan.
Specific Aims: In Aim 1 we will study the effects of PPN-DBS using clinical scales, such as the PSP Rating Scale (PSPRS), the Unified Parkinson's Disease Rating Scale (UPDRS) and the PSP Quality of Life scale. In Aim 2 we will analyze the effects of PPN-DBS in gait initiation, spatiotemporal characteristics of gait and postural sway. In Aim 3 cerebral blood flow (rCBF) changes in cortical and subcortical regions will be studied with and without PPN-DBS using PET imaging.
Study design: Six PSP patients with prominent axial motor signs (gait and balance impairment) will be enrolled. Patients will be assessed before and after surgery (at 3, 6 and 12 months) using the PSPRS, the UPDRS and the PSP QoL questionnaire. Assessments will be performed with and without dopaminergic medications and with and without PPN-DBS. Neuropsychological and psychiatric assessments will be performed preoperatively and at 6-month follow-up. Patients will also have gait analysis and posturography study before and after surgery (with and without stimulation). All patients will have unilateral PPN-DBS surgery using MRI and intraoperative microrecording for targeting. Patients will also undergo rCBF PET scan imaging with and without stimulation.
PSP relevance: PPN-DBS could be an effective surgical treatment and improve gait, postural disturbances and quality of life in PSP patients.

The Long Latencies of ALS, Parkinsonism and Dementia on Guam
John C. Steele, MD, Guam Memorial Hospital

Objective; To define the latencies of ALS/PDC.
Background;Until 1960, native Chamorros of Guam and migrants of other ethnicity were prone to a familial proteinopathy which accumulated tau, alpha-synuclein, ubiquitin and TDP-43 (McGeer) and that was expressed by phenotypes of classical ALS, parkinsonism including PD, PSP and CBD, and dementia of Alzheimer and frontotemporal type (Steele).
It is termed the ALS/Parkinsonism-dementia complex (ALS/PDC) and is known locally as lytico-bodig.

During the past 50 years ago, the phenotypes of ALS/PDC have steadily declined and the age in onset has increased. In 2009, ALS which was 100 times more common than elsewhere in 1953 is now rare, parkinsonism is much less frequent, and dementia is the only phenotype which remains prevalent in late life.
Methods; We have learned about the latencies of the different phenotypes of ALS/PDC by studying them in Chamorro military migrants from Guam, and those of other ethnicity to the island.
Results; Chamorros who were asymptomatic when they left Guam in military service before 1965 and lived abroad subsequently developed neurological symptoms many years later. The intervals were different and longer for parkinsonism than ALS, and longest for dementia.
Majoor-Krakauer has reported that US servicemen residing on Guam for only 1 to 2 months at the end of WW II developed ALS 35 years later, and pathologists Hirano and Perl have identified resident Caucasians coming to Guam in 1952 and 1956 who developed fulminant ALS with NFD 41and 39 years later.
After 1947, Filipinos came as construction workers to rebuild Guam and many remained as residents. Garruto has confirmed pathologically verified ALS with NFD and PDC in them after long latencies that are similar to those of Chamorros.
The longest interval so far recorded is in a 17 year old Filipino immigrant to Guam in 1947 whose symptoms of parkinsonism and Richardson's syndrome did not begin until 55 years later when he was 72 years of age. His certain diagnosis of ALS/PDC is confirmed by neuropathologist Dickson.
Conclusions; Our observations explain the manner and reasons for the orderly decline of ALS/PDC by the latency of its phenotypes after the ending of its cause on Guam in the 1950s.
Although, the etiology of ALS/PDC is still not identified, its long latency is similar to chronic cerebral infections including postencephalitic parkinsonism, SSPE and prion disease, and suggests a similar pathogenesis.

Phenothiazine-mediated Rescue of Cognition inTauTtransgenic Mice requires Neuroprotection and ReducedSoluble Tau Burden, John C. O'Leary III, MD, University of South Florida

The pathological accumulation of tau has generally been thought to facilitate neurodegeneration and lead sequentially to intellective deterioration. Present evidence, on the other hand, suggests that tangles may not be the quintessence of tau that is behind memory loss; rather an intermediate tau species. For this reason, efforts to discover therapeutics for tauopathies are concentrated on the reductions of soluble tau as well as neuroprotection. Here, we found that neuroprotection alone caused by methylene blue (MB) was not sufficient to rescue cognition in a mouse model of human tauopathy. However, only when the protein levels of soluble tau were jointly reduced (by a very high concentration of MB) was cognitive improvement observed. This shows that neurodegeneration can be decoupled from tau accumulation. Also, it may be that phenotypic improvement in tauopathies is only possible when solube tau levels are also reduced. If this is the case, the development of neuroprotective agents may ultimately be an ineffective approach to tauopathies if the soluble toxic tau intermediates are not also reduced. As a result, the properties of MB may converge synergistically to allow for the survival and proper function of neurons and thus allowing it to be a promising therapeutic candidate. In conclusion, these data suggest that neuroprotection can be achieved without reducing soluble tau levels, which is indicative of a molecular link between tau and cell death cascades. Moreover, these signaling cascades could potentially be a therapeutic target in tau related disorders.

Effect of Haplotype-Sequence Variation on the Expression of Alternatively-Spliced MAPT Exons. Tara M. Caffrey, DPhil, University of Oxford

Two major haplotypes have been defined spanning the microtubule associated protein tau (MAPT) genomic locus, H1 and H2, of which H1 is associated with progressive supranuclear palsy. Despite

consistent and strong association of the H1 haplotype with tauopathies, the mechanisms by which H1 polymorphisms confer susceptibility to neurodegeneration have not been defined.
In previous work published from our laboratory, we have shown that H1 expresses 40% more exon 10+(4R) MAPT than H2, suggesting a mechanism behind the association of H1 with PSP, a 4R tauopathy. More recently, we have shown that H2, the protective haplotype, expresses twice as much exon 2+3+ MAPT, from which we propose exon 3 inclusion may have a protective effect. We are now investigating the sequence variation within the MAPT haplotypes on the regulation of alternative splicing of exons 3 and 10. In our current work, we aim to identify the specific sequence variants responsible for the differences in the expression of alternatively spliced MAPT exons between the H1 and H2 haplotypes.
We have engineered MAPT H1 and H2 expression vectors containing the entire MAPT genomic locus. These vectors contain 143 kb of MAPT sequence including 10 kb of upstream promoter. The H1 and H2 vectors are identical in sequence except for at sites of haplotype-variation. We have also created pairs of "haplotype-hybrid" expression vectors by excising polymorphisms from H1 and inserting these into H2. In order to assay haplotype-specific expression of the alternatively spliced exons 3 and 10 in human neuronal cell culture, we have engineered tags into the MAPT vectors. To assay expression of exons 3 and 10, we have introduced an HA-tag and a YFP-fusion tag respectively. This collection of MAPT expression vectors provides an excellent platform to probe for the effect of haplotype-sequence variation on the expression of the alternatively spliced exons at the MAPT locus.

WGA: Study Rationale and Methods
Hakon Hakonarson, MD, PhD, University of Pennsylvania

Abstract:
Progressive supranuclear palsy (PSP) is a rare brain disorder with onset in late adulthood manifesting in deterioration of gait, eye movement and balance. GWAS is a robust approach to identify variants that predispose to complex genetic disorders. To identify single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) increasing risk of PSP, we performed a whole-genome SNP and CNV analysis on a cohort of 2165 PSP cases and 6847 controls of European ancestry who were genotyped with 660 thousand probes. The GWAS method will be discussed, including the QC process performed on the dataset and the CNV approach/analysis will be discussed and some findings highlighted

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GWAS in Progressive Supranuclear Palsy: Statistical Analyses and Results
Nadine Melhem, PhD, University of Pittsburgh School of Medicine, Pittsburgh, PA

Abstract:
The rationale and an overview of the study methods have been presented. Here, we will focus on the statistical analyses conducted and the study results.
Statistical Analyses: We conducted a two-stage genome-wide association (GWA) study of Progressive Supranuclear Palsy (PSP). The Stage 1 sample consisted of 1,114 subjects diagnosed with autopsy-confirmed PSP and 3,287 controls. The Stage 2 sample consisted of 1,051 PSP cases and 3,560 controls, all independent of Stage 1 cases and controls.
QC for association analyses was performed at the level of the individual and then SNP level. Cases and controls were matched based on genetic distances derived from Spectral-Gem to control for genetic ancestry. At Stage 1, we analyzed 531,451 single nucleotide polymorphisms (SNPs) and examined their association with PSP using conditional logistic regression and a log-additive model. At Stage 2, 4,099 SNPs were analyzed. These SNPs were selected from the Stage 1 analyses as SNPs showing association by the log-additive or non-additive models at a p-value ≤ 0.001. Joint analysis of Stages 1 and 2, following *Skol et al.*'s method, was conducted and markers considered genome-wide significant were those that meet the threshold for GWA-significance from the joint analysis. Exploratory analyses including corticobasal degeneration (CBD) cases, examining dominant and recessive models, and evaluating gene-gene interactions in SNPs with the smallest p-values were conducted. We then examined whether SNPs associated by joint analysis had an impact on the expression of genes proximate to them. Gene

expression was assessed in tissues from the cerebellum and the frontal cortex obtained from a sample of 387 neurologically normal Caucasian subjects. DNA samples from these subjects were also genotyped using the Illumina genotyping arrays.
Results: SNPs in *STX6* at 1q25.3, *MOBP* at 3p22.1, and the approximately 1-Mb region containing *MAPT at* 17q21.31 showed genome-wide significant associations with PSP at Stage 1 and the joint analysis. In the *MAPT* region, rs242557 remained associated with PSP even after controlling for the inversion/non-inversion H1/H2 polymorphism. SNPs falling in or near *MOBP* have an effect on *MOBP* expression, but are more strongly correlated with *SLC25A38* expression in the cerebellum, a gene 70 kb away from *MOBP*. In addition, SNPs across the *MAPT* region show strong correlation with *MAPT*, *ARL17A*, *PLEKHM1*, and *LRRC37A4* expression, which disappears after controlling for H1/H2 polymorphism except for *ARL17A* expression. However, we find that the risk for PSP does not rise and fall with *ARL17A* expression.
The biological interpretation of these findings and their implications on our understanding of the etiology of PSP will follow.

Tau, the H1 Haplotype and Gene Expression
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Abstract:
The tau gene and protein continues to hold centre stage in PSP pathogenesis; four-repeat tau (4R-tau)-dominant neuronal and glial tau inclusions define the PSP brain and the recent genomewide association study (GWAS) has cemented the role of the *MAPT* H1 haplotype (*MAPT*-H1) as the strongest genetic risk factor. Underlying variation within the H1 haplotype in the form of the H1c sub-haplotype is the main driver of this risk and is associated with increased haplotype-specific *MAPT* transcription as well as increased production of 4R-tau mRNA. This is consistent with the pathological picture of PSP and presents a plausible mechanism in view of related findings on the APP gene in Alzheimer's disease and the alpha-synuclein gene (SNCA) in Parkinson's disease (PD), whereby common risk variants or gene multiplications have the effect of increasing levels of the fibrillogenic substrates that form the defining pathogenic inclusions of these disorders. Interestingly, together with *SNCA*, *MAPT*-H1 is the strongest risk factor in sporadic PD. Do increased levels of tau sensitise vulnerable neurons in PD without the necessary formation of tau inclusions?
The inversion on chromosome 17 that causes the H1/H2 dichotomy contains several other genes, some of which have important functions in neurons. The recent GWAS has shown that most, if not all the genes in this region show haplotype-specific expression differences, possibly due to positional effects of the inversion. Although tau pathology in PSP makes *MAPT* the most obvious suspect, we should by no means exclude the possibility of involvement of the other genes. However, conditional analysis based on the H1/H2 dichotomy strongly implicates the rs242557 SNP in the promoter region of *MAPT.* This SNP that defines H1c is in a conserved transcriptional repressor region, and has a strong allelic effect on *MAPT* promoter strength. It is a distinct possibility that promoter variation alone could explain the risk with co-transcriptional regulation of splicing and that *MAPT* gene expression could be affected by a combination of the *cis*-effects of gene polymorphisms and the positional effects of the H1/H2 inversion with the latter havinge dramatic effects not only on chromatin context but also higher-level gene regulatory mechanisms.

GWAS in PSP: Results at disease-associated loci other than MAPT
Ulrich Müller, MD, PhD, Justus Liebig University

Abstract:
A two-stage GWAS was performed in PSP. Stage 1 included 1,114 autopsy-confirmed cases, stage 2 comprised 1,051 mostly clinically defined patients. All stage 1 samples were genotyped on Human 660W-Quad Infinium Beadchips. Stage 2 samples were genotyped for all 4,099 SNPs that indicated association at $p \leq 0.001$ in stage 1. In total, genotyping results at 531,451 SNP loci analyzed in stage 1 were compared with those obtained in 3,287 controls. Associations obtained at stage 1 and stage 2 were analyzed separately as well as jointly. All three analyses resulted in genome-wide significant association levels of $p < 5x10^{-8}$ at locus MAPT. At an additional 5 loci common variants associated with PSP were detected at

stringent significance levels. These included regions on chromosome 1, 2, 3, and 6. The location of the associated SNPs suggests functional relevance in PSP of genes in these regions, i.e. STX6 (syntaxin 6) at 1q25.3, EIF2AK3 (translation initiation factor 2α kinase 3) at 2p11.2, MOBP (myelin-associated oligodendrocytic basic protein) at 3p22.1, and IRF4 (Interferon regulatory factor 4) at 6p25.3. Analysis at a slightly lower significance level implicates the additional genes MR1, CD8B, IL2/IL21, EXOC2, BMS1, SLCO1A2, and NELL2 in PSP. The most significant genes are connected with the ER (EIF2AK3) and Golgi apparatus (STX6) and point to a role of disturbed ER- and Golgi- function in the etiology of PSP. The other significantly associated genes, i.e MOBP and IRF4, are required for myelin formation and immune response, respectively. Of the less significantly associated genes, several (CD8B: cluster of differentiation 8; IL2/Il21: interleukin 2 and 21; MR1: major histocompatibility complex class I–related molecule) indicate immunological disturbance in the etiology of PSP. Taken together the findings suggest involvement of several genes in PSP that function in the immune response or within the ER/Golgi system. In addition several additional genes with unrelated functions may play a role in PSP. These include MOBP (potentially myelin formation and function), BMS1 (ribosome assembly), SLCO1A2 (sodium independent organic anion transport), and NELL2 (potential receptor molecule of neurons). The findings do not reveal a common pathway relevant to PSP. It remains to be seen whether the concerted dysfunction of several of these genes by either endogenous or exogenous causes is a prerequisite for development of PSP.

GWAS in PSP: Study rationale and methods
Günter Höglinger, MD, Philipps-Universität

In rare autosomal dominant variants of PSP, mutations of the MAPT gene encoding the microtubule-associated protein tau have been reported. Despite these few cases, PSP is a predominantly sporadic disease. However, inheritance of two copies of the H1 haplotype (H1/H1) covering the MAPT gene is a major genetic risk factor for the tauopathies PSP and CBD. Calculations of population attributable risk suggest that only ~68% of the risk for PSP can be accounted for by the MAPT H1 haplotype, suggesting there may be additional risk factors involved in PSP. Therefore, we undertook a genome-wide association study (GWAS), an unbiased, hypthesis-free scan of the entire genome to identify regions harboring PSP risk genes. We performed a 2 stage analysis to maximize efficiency while maintaining power.
Sample collection:
Healthy Controls were recruited within the CHOP Health Care Network, including four primary care clinics and several group practices and outpatient practices that included well child visits.
For Stage 1 we studied only autopsied cases, thereby essentially eliminating incorrect diagnoses, because neuropathologic findings are definitive for the diagnosis of both disorders. We collected DNA from 1,114 autopsy-confirmed cases and 3,287 controls We also studied CBD using a relatively small sample of cases (n = 154).
For Stage 2, we collected DNA from 1,051 subjects diagnosed with PSP and 3,560 control subjects, all independent of Stage 1 cases and controls. The new cohort consisted predominantly of living cases. We used clinically diagnosed PSP cases in this stage because cases diagnosed using clinically-defined criteria are reasonably concordant with autopsy results. All fulfilled the clinical diagnosis of possible or probable PSP. We did not use clinically defined CBD cases because the clinical diagnosis is less reliable than for PSP.
Genotyping:
Healthy Controls were genotyped at the Center for Applied Genomics at CHOP using the Illumina Human HapMap550 Infinium BeadChip, containing 561,724 markers for SNP genotypes.
Stage 1: Cases were genotyped by the Center for Applied Genomics at Children's Hospital at Philadelphia (CHOP) using Human 660W-Quad Infinium Beadchips. We assessed association between genotypes at 531,451 single nucleotide loci. To control for potential confounding due to variation in genetic ancestry, cases and controls were matched based on genetic distances derived from Spectral-GEM using 12,644 SNPs with a > 99.9% call rate and a between SNP distance of > 0.2Mb. The data to be analyzed consisted of 1,114 PSP cases and 3,287 controls. Single nucleotide polymorphisms (SNPs) and disease status using an additive genetic model.
Stage 2: We assessed 4,099 SNPs for association with the disease status. The SNPs were selected from the original set if they yielded a P < 10-3 for any Stage 1 association. Controls and cases were matched for genetic ancestry. Statistical analysis:

For the joint analysis of Stage 1 and 2 results (z-values) we interpreted significance at three levels:
-Stringent (P < 5x10-8),
-Bonferroni-corrected (1x10-7, roughly the number of independent tests), and
-False Discovery Rate (3.2x10-7), expected to produce a false discovery rate of 1 in 20.
Expression Quantitative Trait Loci (eQTL) Analysis:
Tissues from the cerebellum (CRBLM) and frontal cortex (FCTX) were obtained from N=387 neurologically normal Caucasian subjects. Genotyping was performed from the cerebellum tissue sample using the Illumina HumanHap550 v3, Human610-Quad v1 and Human660W-Quad v1 Infinium Beadchips. Expression profiling for mRNA transcripts was assayed using the Illumina HumanHT-12 v3 Expression Beadchip. eQTL analysis was performed using residuals for every trait in both brain tissues using mach2qtl to regress the trait with the allele dosage. The PSP Stage 1, 2 and Joint analysis associated variants were then checked against these eQTL results.
Concluding remarks:
In sum, this study was designed and performed according to the state of the art to identify genetically determined risk factors for PSP.

Haplotype regulation of alternative splicing at the MAPT locus
Richard Wade-Martins, DPhil, University of Oxford

Abstract:
In the post-GWAS era it is becoming clear that identifying functional polymorphisms underlying genetic association with disease is a major challenge. Previously in H1/H2 heterozygous human brain tissue we have found that the H1 haplotype expressed 40% more exon 10+(4R) MAPT transcript than H2. We also found that the H2 haplotype expressed twice as much exon 2+3+ MAPT transcript than H1. These data suggest a mechanism for the susceptibility of H1 carriers to PSP. We are now using a unique genomic DNA expression system to functionally dissect variation across the MAPT genomic DNA locus. Infectious vectors are an efficient means of delivering genes to cells, but the size of most genomic loci generally precludes their use in the context of viral constructs. Our laboratory has pioneered an efficient viral delivery and expression system for genomic DNA loci >100 kb in size, which we have termed the infectious bacterial artificial chromosome, or iBAC. The iBAC is based on the herpes simplex virus type 1 (HSV-1) amplicon vector, an exciting tool for genomic DNA locus expression because HSV-1 has a high transgene capacity of ~150 kb. We have generated several iBAC-MAPT vectors expressing the H1 or H2 wild-type variants, or carrying FDTP-17 mutations, and demonstrated accurate physiological and developmental regulation of MAPT expression in neurons. We have used homologous recombination in bacteria to generate a number of "haplotype-hybrid" vectors, focusing on polymorphisms around exons 3 and 10. We have swapped over the 238 bp insertion/deletion polymorphism situated 714 bp upstream of exon 10. We have also re-engineered the A(H1)/G(H2) SNP 9 bp downstream of exon 3 (rs1800547). To facilitate analysis of expression from the MAPT genomic locus vectors gene we have also introduced a number of tags into the MAPT transgene, such as GFP, and generated a series of luciferase expression vectors to monitor alternative splicing. We will describe the wide range of new tools we have developed and present our data which suggest that the H1 238 bp insertion enhances inclusion of exon 10.

The Effects of the CBD-Associated Tau Gene H1 Haplotype on Tau Expression
Hana Dawson, PhD, Duke University

Lay Abstract:
A specific subtype of the TAU gene called the H1 haplotype is associated with several DNA changes and occurs more frequently in patients with CBD and PSP than in the general population. Tau is an integral protein that stabilizes the scaffolding that allows for neurons to extend and maintain long processes. Tau binds to the scaffolding with either 3 or 4 regions and the number of binding regions tau contains is determined by developmental and physiological regulation. In CBD and PSP, the ratio of tau protein with 4 binding regions is increased relative to tau protein with 3 binding regions. By expressing a DNA construct

that is able to increase tau protein with 4 binding regions in transgenic mice, we were able to confirm that this increase is causal in neurodegeneration. Furthermore, we were able to determine from these transgenic mice that the region of the human TAU gene that regulates the amount of tau protein expressed, the promoter, also regulates the amount of 3 and 4 binding regions that are expressed. We therefore believe that some of the DNA changes that are associated with the H1 haplotype and are located in the promoter may predispose to PSP. By deleting small sequences in the tau promoter we were able to identify regions that alter the amount of tau protein with 4 binding regions. Five H1 TAU haplotype documented DNA changes lie within these regions. Furthermore, by targeting DNA sequences in the binding region with small DNA molecules we were able to show a relationship between the TAU promoter and these regulatory sequences.

Scientific Abstract:
A variant of the TAU gene called the H1 haplotype is associated with several nucleotide changes (polymorphisms) and occurs in 95% of PSP patients but only in 65% of the general population. One of the roles of tau protein is to regulate the stability and assembly of microtubules by binding to them through 3 or 4 microtubule binding repeats (MTBR). Whether the tau protein has 3 or 4 MTBR is determined by the developmental and physiological regulation of the inclusion or exclusion of the second MTBR (MTBR-2). The H1 haplotype may predispose individuals to PSP by altering the inclusion or exclusion of MTBR-2. First, tau protein in PSP patients primarily includes MTBR-2 while in normal adults only 50% of tau protein includes MTBR-2. Second, TAU gene mutations that increase the inclusion of MTBR-2 are causal in FTDP-17, a neurodegenerative disease that can resemble PSP.
We have generated a tau minigene construct that by design allows for the inclusion or exclusion of MTBR-2 and is under the regulation of the human tau promoter. By expressing the minigene construct with a FTDP-17 mutation in transgenic mice, we were not only able to confirm that increasing the inclusion of MTBR-2 is causal in neurodegeneration but also that the human tau promoter regulates the inclusion or exclusion of MTBR-2 during development. We hypothesized that some of the polymorphisms associated with the H1 haplotype are located in regions that regulate the inclusion/exclusion of MTBR-2 and hence predispose to PSP. By deleting sequences in the human tau promoter of the tau minigene construct we were able to identify promoter regions that alter the splicing of exon 10. Five H1 TAU haplotype documented polymorphisms (three nucleotide changes, a nucleotide deletion and a short sequence insertion) lie within these regions. Furthermore the levels of exon 10 inhibition by small antisense oligonucleotide molecules were influenced by the deletions in the promoter.