

Embargoed until Nov. 14, 12:30 p.m. PST
Press Room, Nov. 12-16: (619) 525-6230

Contacts: Emily Ortman, (202) 962-4090
Kym Kilbourne, (202) 962-4060

Exploring the Role of a Rogue Protein in Parkinson's Disease

Findings point to new therapeutic targets and possible path for stem cell therapies

SAN DIEGO — Research released today highlights the role of alpha-synuclein, a protein that accumulates and spreads in the brains of Parkinson's disease patients over time, offering new insights into the onset, physiology, and treatment options for the disease. The studies were presented at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease, affecting more than 10 million people worldwide. Characterized by "Lewy bodies," clumps of protein composed primarily of alpha-synuclein, the disease gradually destroys brain cells that produce dopamine, a neurotransmitter involved in regulating movement. Over time, patients develop symptoms such as tremor, rigidity, loss of movement control, and ultimately dementia.

Today's new findings show that:

- In mice, clumps of misfolded alpha-synuclein traveled from the lining of the stomach and intestine to the brain, lending support to the theory that Parkinson's may start in the gut (Collin Challis, abstract 468.15, see attached summary).
- Astrocytes, non-neural cells intertwined with nerve cells, may play a role in how alpha-synuclein travels from one brain cell to another (Jinar Rostami, abstract 13.07, see attached summary).
- Dopamine-producing cells derived from fetal tissue can survive for decades without immunosuppression after being implanted the brains of Parkinson's patients, suggesting potential ways to proceed with stem cell therapies created in the lab (Curt Freed, abstract 287.02, see attached summary).
- An experimental drug reduced levels of alpha-synuclein in the brains of mice, suggesting a possible new therapeutic target (Sergio Pablo Sardi, abstract 602.29, see attached summary).

"Many diseases that cause brain cell death share a common feature: a misfolding protein that sets off a chain reaction of abnormal binding and clumping," said press conference moderator Alice Chen-Plotkin of the Hospital of the University of Pennsylvania. "Today's findings illustrate the progress we're making in understanding how the accumulation and spread of alpha-synuclein leads to the death of brain cells that trigger Parkinson's disease and point to new potential therapeutic targets."

The research was supported by national funding agencies such as the National Institutes of Health, as well as other public, private, and philanthropic organizations worldwide. Find out more about Parkinson's disease at BrainFacts.org.

Related Neuroscience 2016 Presentation

Special Lecture: Circuits for Movement
Sunday, Nov. 13, 1-2:10 p.m., SDCC Ballroom 20

Abstract 468.15 Summary

Lead Author: Collin Challis, PhD

California Institute of Technology
Pasadena, Calif.

viviana@caltech.edu

Accumulation of Abnormal Protein in Gut Linked to Onset of Parkinson's Symptoms in Mice

Finding supports theory that Parkinson's disease may start in gut, move to brain

New research in mice tracks the spread of Parkinson's disease-associated proteins in neurons from the gut to the brain, supporting the hypothesis that the ailment might start outside the brain. The study was released today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

The buildup of misfolded alpha-synuclein proteins throughout the brain is a hallmark of Parkinson's disease. However, alpha-synuclein can accumulate in other areas of the body, namely the olfactory bulb and intestines, as demonstrated by autopsy studies of Parkinson's patients. Some researchers suggest that misfolded proteins accumulate in those areas years before reaching the brain.

In this study, researchers explored whether clumps of alpha-synuclein, called fibrils, could spread from the gut to the brain, potentially via the vagus nerve, which is involved in controlling peripheral organs like the liver and the spleen. This idea has been suggested by other studies. The researchers injected a synthetic, misfolded version of alpha-synuclein into the lining of the stomach and intestines of adult mice. For three months researchers collected information on the animals' gastrointestinal health and evaluated their balance, strength, and coordination. To track the spread of the fibrils, they used a technique that makes targeted areas of the body transparent.

The misfolded alpha-synuclein initiated a chain-reaction of toxic accumulation that moved from one body area to the next over a period of 90 days. The misfolded proteins started accumulating in the gut seven days after injection, peaking on day 21 and reaching the brainstem, where the vagus nerve enters the central nervous system. After 60 days, the fibrils were observed in the midbrain, where dopamine-producing cells are found. Accumulation of fibrils correlated with changes in the bowel and declines in animals' ability to move, symptoms seen in Parkinson's patients years before a diagnosis. Control mice saw no such effects.

"Our findings suggest that pathologic alpha-synuclein that initially aggregates in the gut can propagate to the brain in a time-dependent manner," said lead author Collin Challis, a postdoctoral fellow at the California Institute of Technology. "It also supports the concept that PD may have a dormant period marked by non-motor symptoms due to the accumulation of alpha-synuclein fibrils in peripheral organs."

Research was supported with funds from the National Institute on Aging, National Institute of Neurological Disorders and Stroke, Pew Charitable Trusts, and the Heritage Medical Research Institute.

Scientific Presentation: Monday, Nov. 14, 3-4 p.m., Halls B-H

14207. Progression of Parkinson's-like pathology following inoculation of alpha-synuclein preformed fibrils in the gut
C. CHALLIS¹, T. R. SAMPSON¹, B. YOO¹, S. K. MAZMANIAN¹, L. A. VOLPICELLI-DALEY², V. GRADINARU¹
¹Biol. and Biol. Engin., Caltech, Pasadena, Calif.; ²Neurol., Univ. of Alabama at Birmingham, Birmingham, Ala.

TECHNICAL ABSTRACT: The primary hallmarks of Parkinson's disease (PD) include the formation of inclusions composed of insoluble alpha-synuclein (aSyn) fibrils known as Lewy bodies and neurites, the loss of catecholaminergic neurons, and motor impairments. However, emerging findings suggest that a prodromal phase characterized by non-motor symptoms such as gastrointestinal (GI) and olfactory deficits may precede clinical diagnosis of PD (Hawkes et al., 2010). Postmortem biopsies from asymptomatic and PD-diagnosed individuals revealed the presence of pathologic aSyn assemblies in GI and olfactory tissue, suggesting propagation of aSyn fibrils from peripheral organs to the central nervous system where they precipitate PD. This is supported by findings that aSyn fibrils are interneuronally transported and can seed the formation of additional fibrils from endogenous aSyn monomers (Volpicelli-Daley et al., 2011). Here, we inoculated stomach and duodenum lining of adult C57Bl/6 mice with aSyn preformed fibrils (PFF) and assessed GI health, motor function, and histology at 7, 21, 60, and 90 days post-PFF injection (dPPI) compared to saline-injected controls. At 7 and 21 dPPI, fecal composition and output was significantly altered, with measures returning to baseline levels by 60 dPPI. Using a battery of tests of motor strength and coordination we found significant deficits in the inverted wire hang and adhesive removal tasks at 60 and 90 dPPI. We performed PACT (PAssive CLARITY Technique) on whole, intact stomach and duodenum as well as 500µm thick brain sections and stained for phosphorylated (P)-aSyn, a pathological modification of aSyn fibrils not present on PFF. In the gut, fibril formation increased at 7 dPPI and peaked at 21 dPPI in enteric neurons of the villi, crypts, and submucosal and myenteric plexuses, with signal diminishing to baseline levels at 60 dPPI. At 21 dPPI we observed an increase in P-aSyn signal in vagal nuclei of the brainstem and at 60 dPPI, P-aSyn was observed in the midbrain. Our results indicate time-dependent aSyn fibril propagation from the gut to the central nervous system via the vagus nerve following gut PFF inoculation that triggers early GI dysfunction and subsequent motor deficits.

Abstract 13.07 Summary

Lead Author: Jinar Rostami

Uppsala University
Uppsala, Sweden

jinar.rostami@pubcare.uu.se

Astrocytes May Play Role in Spreading Misfolded Protein Associated With Parkinson's *Findings in cell culture show astrocytes passing protein clumps from cell to cell*

Astrocytes, star-shaped cells that support neurons and defend them from infection and injury, may play a role in the spread of misfolded proteins associated with Parkinson's disease, according to research released today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

In Parkinson's disease, buildup of misfolded protein alpha-synuclein in and around neurons precedes the death of those neurons. Previous studies indicate clumps of misfolded alpha-synuclein initiate a chain reaction of abnormal folding, moving from cell to cell and inducing normal proteins nearby to misfold. However, the mechanisms behind this cellular hand-off has remained a mystery.

In this study, researchers examined how astrocytes grown from human embryonic stem cells process normal and misfolded alpha-synuclein. The team exposed astrocytes to normal or misfolded proteins for 24 hours to incorporate the proteins and then grew the cells in a culture dish. After six days, astrocytes exposed to misfolded protein showed signs of distress: Clumps of protein, or Lewy bodies, accumulated in the cell's inner compartments, including cellular regions that synthesize, fold, and modify proteins. In addition, energy-producing mitochondria faltered. Moreover, misfolded protein appeared to spread from cell to cell.

"Our findings highlight, for the first time, the role of astrocytes in the propagation of abnormal alpha-synuclein, potentially reflecting the disease process in the brains of patients with Parkinson's disease," said Jinar Rostami, a doctoral student in Anna Erlandsson's group at Uppsala University in Sweden. The group now plans to see whether astrocytes also transfer misfolded proteins to neurons.

Research was supported with funds from the Swedish Research Council, the Parkinson Foundation, the Alzheimer Foundation, Åhlén Foundation, Hedlunds Foundation, Crafoord Foundation, Åke Wiberg Foundation, Kockska and Zoegas Foundations.

Scientific Presentation: Saturday, Nov. 12, 2:30-2:45 p.m., SDCC 5B

4371. Accumulation and spreading of alpha-synuclein oligomers in human astrocytes

J. ROSTAMI¹, S. HOLMQVIST², V. LINDSTRÖM¹, J. SIGVARDSON³, M. INGELSSON¹, J. BERGSTRÖM¹, L. ROYBON², A. ERLANDSSON¹

¹Publ. Health and Caring Sciences, Molecular Geriatrics, Uppsala Univ., Uppsala, Sweden; ²Dept. of Exptl. Med. Sci., Lund Univ., Lund, Sweden; ³BioArctic Neurosci. AB, Stockholm, Sweden

TECHNICAL ABSTRACT: Parkinson's disease (PD) is characterized by intracellular protein inclusions called Lewy bodies, composed mainly of aggregated alpha-synuclein. Although, alpha-synuclein deposits are primarily found in neurons, they also appear frequently in glial cells at advanced disease stages. Many lines of evidence suggest a prion-like propagation of aggregated alpha-synuclein during PD progression, but the cellular mechanisms behind the spreading is still elusive. The aim of this study was to clarify the role of astrocytes, the most prominent glial cell type, in alpha-synuclein propagation. For this purpose, human astrocytes, derived from embryonic stem cells were treated with Cy3-labelled monomeric or oligomeric alpha-synuclein for 24 h. Following exposure, the cells were thoroughly washed and incubated for 0, 3 or 6 days in alpha-synuclein free medium. Using time lapse microscopy and immunocytochemistry we investigated intracellular localization, toxic effects and spreading of alpha-synuclein between the astrocytes. Our results demonstrate that human astrocytes engulfed both alpha-synuclein oligomers and monomers. Interestingly, we found that the monomers were quickly degraded, while the oligomers accumulated in the astrocytes. By immunocytochemistry, we showed that the accumulated alpha-synuclein oligomers co-localized with the lysosomal marker, LAMP-1, at the earliest time point. However, the co-localization disappeared over time, leaving large deposits of alpha-synuclein in the astrocytes, indicating an incomplete digestion. Staining with specific markers for various organelles revealed that the alpha-synuclein deposits localize to the region of the trans-golgi network. Furthermore, a clear effect of the oligomer exposure was observed on mitochondria homeostasis which was also confirmed by electron microscopy. In addition, time-lapse recordings demonstrated frequent cell-to-cell transfer of alpha-synuclein inclusions in the astrocyte culture. Interestingly, the astrocytes appeared to use different spreading mechanisms, depending on the size of the inclusions that were transferred. In summary, our results identify a possible role of astrocytes in the spreading of alpha-synuclein pathology.

Abstract 287.02 Summary

Lead Author: Curt Freed, MD

University of Colorado Health Sciences Center
Denver

(303) 724-6002
curt.freed@ucdenver.edu

Dopamine-Cell Transplants Survive Without Immunosuppression for Lifetime of Parkinson's Patients

Research suggests possible path forward with cell transplants created from embryonic stem cells

Drugs to suppress the immune system may not be necessary for Parkinson's disease therapies based on embryonic stem cell transplantation, according to research released today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

In an effort to supplement the dopamine deficits that cause tremors and rigidity, doctors at the University of Colorado School of Medicine implanted human dopamine-producing fetal cells into the brains of 61 patients with advanced Parkinson's disease from 1988 to 2000. The transplants couldn't cure the disease, but the researchers hoped to offer symptomatic relief as a substitute to treatment with L-dopa. As a result, the physicians decided against using immunosuppressive drugs that could place patients at risk for infections.

In this study, researchers examined the brains of 16 of those patients who died seven months to 27 years after having the transplant surgery. They found healthy dopamine-producing neurons resided in each patients' brain at death and many of the transplanted neurons had developed normally, making connections with other neurons in areas outside the transplant region. More importantly, the immune system had not attacked or destroyed the transplanted cells.

"Every fragment of fetal tissue that we put into every person has shown surviving dopamine neurons, providing evidence that transplanted dopamine cells can survive and function, even in the absence of immunosuppressants," said Curt Freed, director of the Neurotransplantation Center for Parkinson's Disease at the University of Colorado who participated in both the transplants and autopsy studies. "Moving forward with treatments developed from embryonic stem cells, it's unknown whether there will be that same lack of immune response. Still, these findings suggest that those transplants could proceed without immunosuppression to see whether the cells are accepted as the fetal cells have been."

Research was supported with funds from Robert and Charles Stanton, the National Institute of Neurological Disorders and Stroke, and personal funds of Curt R. Freed, MD.

Scientific Presentation: Monday, Nov. 14, 8:15-8:30 a.m., SDCC 1B

14693. Transplants of human fetal dopamine neurons into putamen of Parkinson patients survive for at least 27 years without immunosuppression

C. R. FREED¹, R. E. BREEZE², B. A. SYMMES¹, S. FAHN³, D. EIDELBERG⁴, W. ZHOU¹

¹Div. of Clin. Pharmacol., ²Neurosurg., Univ. of Colorado, Aurora, CO; ³Neurol., Columbia University/Weill Cornell Med. Ctr., New York, NY; ⁴Ctr. for Neurosci., Feinstein Inst. for Med. Res., Manhasset, NY

TECHNICAL ABSTRACT: We have shown that fetal dopamine cell transplants significantly improve objective signs of Parkinson's disease in direct relation to the preoperative response to L-dopa. From our total of 61 patients transplanted between 1988 and 2000, we have had the opportunity to study postmortem brain of 16 subjects who have died 7 months to 27 years after transplant. Dopamine neurons were identified immunohistochemically in transplant tracks using a primary antibody to tyrosine hydroxylase and a secondary antibody labeled with fluorescent FITC. After identification of dopamine neurons by green fluorescence, pigment density was determined by measuring white light transmission through the cytoplasm of each cell. Twenty-five cells in each transplant track were measured. Pigment density increased linearly in transplanted dopamine neurons during the years after transplant. Parallel studies were performed on substantia nigra of children and adults who did not have Parkinson's disease. A similar age-related accumulation of pigment was seen. Despite the absence of immunosuppression at the time of surgery and in subsequent years, every fragment of human embryonic mesencephalon showed surviving dopamine neurons, indicating that the immune system has not destroyed any transplant. While a few isolated Lewy-body like inclusions were occasionally observed, all transplants showed extensive fiber outgrowth into striatum, indicating that the transplanted dopamine neurons were physiologically robust. The number of surviving dopamine neurons was not related to the time since transplant, indicating there is no progressive loss of neurons with time. The largest number of surviving dopamine neurons was found in a patient who died at age 85, ten years after transplant at age 75, indicating that age is not a factor affecting survival of dopamine neurons. We conclude that transplanted human dopamine neurons undergo morphologic evolution essentially identical to that seen in the normal substantia nigra. This result suggests that the neurotrophic environment of the putamen of patients with idiopathic Parkinson's disease is normal since it can support long term development of transplanted dopamine neurons.

Abstract 602.29 Summary

Lead Author: Sergio Pablo Sardi, PharmD, PhD
Sanofi Genzyme
Framingham, Mass.

Media Contact: Lisa Clemence, (617) 768-6699
lisa.clemence@genzyme.com

Studies in Mice Show Experimental Drug Reduces Buildup of Protein Associated With Parkinson's Disease *Research may lead to new therapies for decreasing accumulation of alpha-synuclein in the brain*

A drug that curtails production of specific lipids in the brain also reduces the amount of a Parkinson's disease-associated protein in the brain, according to research in mouse models released today at Neuroscience 2016, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

The most common genetic risk factor for Parkinson's disease involves a defect in the *GBA* gene, affecting 5 percent to 10 percent of patients with the disease. *GBA* encodes for an enzyme that breaks down lipids known as glucocerebrosides. Defects in *GBA* and consequent loss of enzymatic activity allow glucocerebrosides to build up in cells. While the accumulation of lipids is toxic, causing enlarged spleens and weakened bones in patients with Gaucher disease, it doesn't cause Parkinson's directly. However, harboring even one copy of mutant *GBA* in your DNA increases the risk of developing Parkinson's disease.

In this study, researchers tested an experimental drug, GENZ-667161, which inhibits the production of glucocerebrosides, hypothesizing that producing fewer lipids may counteract the challenge of clearing them from the cells. In mice with mutant *GBA*, administration of GENZ-667161 over a period of eight months reversed the accumulation of glucocerebrosides in the brain. These mice also contained lower levels of aggregated alpha-synuclein, a protein that builds up in the brains of people with Parkinson's disease, and had improved memory compared with control mice. Follow-up studies demonstrated that mice harboring wild type *GBA* but genetically engineered to accumulate alpha-synuclein also benefited from administration of GENZ-667161, showing reduced overall alpha-synuclein levels.

"The findings suggest a possible treatment strategy for Parkinson's patients who have mutations in the *GBA* gene," said lead author Sergio Pablo Sardi of the Neuroscience Research Therapeutic Area at Sanofi Genzyme, the specialty care global business unit of Sanofi. The company now plans to evaluate a glucocerebroside inhibitor in clinical trials.

This research was supported by Sanofi.

Scientific Presentation: Tuesday, Nov. 15, 1-2 p.m., Halls B-H

Abstract 16034. Scientific Abstract – Inhibition of glucosylceramide synthase alleviates aberrations in synucleinopathy models
S. SARDI, C. VIEL, J. CLARKE, A. RICHARDS, H. PARK, J. DODGE, J. MARSHALL, B. WANG, S. CHENG, L. S. SHIHABUDDIN;
Sanofi Genzyme, Framingham, Mass.

TECHNICAL ABSTRACT: Mutations in *GBA*, the gene encoding glucocerebrosidase, are associated with an enhanced risk of developing synucleinopathies such as Parkinson's disease (PD). Recent studies have also demonstrated that genetic variation in *GBA* can impact the progression of PD. Patients harboring mutations in *GBA* present higher prevalence and severity of motor and non-motor symptoms. However, the precise mechanisms by which mutations in *GBA* increase PD risk and exacerbate its progression remain unclear. Here, we investigated the merits of glucosylceramide synthase (GCS) inhibition as a potential treatment for synucleinopathies. A Gaucher-related synucleinopathy mouse model (GbaD409V/D409V) was treated with an orally available brain-penetrant GCS inhibitor, Genz-667161 for 8 months. This intervention prevented CNS substrate lipid accumulation. Most notably, treatment with the GCS inhibitor slowed the accumulation of hippocampal aggregates of α -synuclein, ubiquitin and tau, and improved the associated memory deficits. The effects of the GCS inhibitor were also studied in a mouse model overexpressing α -synuclein, PrP-A53T-SNCA, and harboring wild type alleles of *GBA*. Treatment of PrP-A53T-SNCA mice with Genz-667161 for 6.5 months reduced membrane-associated α -synuclein in the CNS and ameliorated cognitive deficits. Collectively, the data indicate that inhibition of GCS can modulate processing of α -synuclein and reduce various α -synuclein entities, thereby reducing the progression of synucleinopathies in mice with and without mutations in *GBA*. The present study provides supporting evidence for the clinical development of brain-penetrant GCS inhibitors in PD and other synucleinopathies.