RESEARCHERS REPORT NEW WAYS TO PREDICT THE DEVELOPMENT OF ALZHEIMER'S DISEASE

Possible New Saliva Test Reported at Alzheimer's Association International Conference 2015

WASHINGTON, DC, July 19, 2015 – Brain scans, memory tests and body fluids such as saliva may hold the keys to understanding a person's likelihood of developing Alzheimer's, even among those who don't yet have memory and thinking problems associated with the disease, suggest four studies reported at the Alzheimer's Association International Conference® 2015 (AAIC® 2015) in Washington, D.C.

Two of the studies suggest that an excess of certain proteins in cerebrospinal fluid (CSF) are strong predictors of Alzheimer's, and that the accuracy of these predictions is stronger when they are considered together with other diagnostic tools, such as memory tests or MRI brain scans. A third report suggests that new ways of creating images of inflammation in the brain with PET scans could one day be used to identify treatments that protect the brain. Finally, a small but intriguing study suggests it could be possible to detect Alzheimer's-like changes in saliva, which is easily obtained, safe and affordable, but there is a lot of work still to be done.

"There is now consensus that Alzheimer's disease begins with changes in the brain that are happening while people are still cognitively normal, decades before memory and thinking problems begin, which then accelerate as the disease progresses," said Maria Carrillo, PhD, Alzheimer's Association Chief Science Officer. "Still, diagnosis of Alzheimer's usually happens fairly late in the progression of the disease, typically not until symptoms are severe enough to prompt a visit to the doctor."

"Earlier diagnosis or, better still, the ability to predict the onset of Alzheimer's, would significantly increase the window of opportunity a person with Alzheimer's has to formulate an informed response to the news and empower them to be an active participant in decision-making while they still have the ability. It also would help researchers choose participants for treatment research, especially people at the earliest, pre-symptomatic stages of the disease who are needed for prevention trials," Carrillo said.

The Alzheimer's Association *2015 Alzheimer's Disease Facts and Figures* report found that only 45 percent of people diagnosed with Alzheimer's or their caregivers say they were told the diagnosis by their doctor. Research suggests that one of the reasons doctors do not disclose a diagnosis of Alzheimer's is they have insufficient time and resources to provide support to patients and caregivers at the time of the diagnosis. Extending the time between diagnosis and the onset of symptoms could change that situation.

Six Factors May Identify People Most Likely to Get Alzheimer's Disease

Research suggests that certain biological changes take place over time that signal the future onset of Alzheimer's disease symptoms in people with otherwise normal memory and thinking skills. Understanding which changes are most predictive of Alzheimer's may help in the selection of candidates for clinical trials and help monitor an individual's response to treatment or prevention strategies.

Most Alzheimer's clinical trials have been conducted among individuals in the dementia phase of the disease. More recently, trials have been initiated during an earlier phase, known as mild cognitive impairment (MCI), where people have a significantly increased risk of progressing to Alzheimer's disease dementia. For more than a decade, these trials have been unsuccessful in identifying new Alzheimer's medications. A few clinical trials have recently been initiated among individuals in the preclinical phase of the disease. However, there is limited data on which to base the selection of individuals for a clinical trial in the preclinical phase; there is a great need for better tools for earlier detection and cognitive assessment to identify these individuals and track their progress.

To assess the usefulness of different predictive tests, Marilyn Albert, PhD, Director of the Division of Cognitive Neuroscience in the Department of Neurology at Johns Hopkins University School of Medicine and Director of the Johns Hopkins Alzheimer's Disease Research Center and colleagues repeatedly evaluated the mental status of 189 participants from the BIOCARD Study who were cognitively normal at the beginning of the study, They used a variety of measurement tools and identified those tools that best predicted the onset of MCI five years later. They found that the combined results of six measures were particularly useful:

- Two memory and thinking tests (the Digit Symbol and Paired Associates Immediate Recall tests).
- Levels of two different proteins in CSF (amyloid beta and p-tau).
- Two MRI brain scans – one to assess the thickness of the right entorhinal cortex and another to measure the volume of the hippocampus, both of which are important for memory.

Several statistical measures were used to characterize the accuracy of prediction, including the Area Under the Curve (AUC), sensitivity and specificity (AUC=0.886, sensitivity = 0.85, specificity = 0.70).

"Our study shows that – up to five years before any Alzheimer's symptoms appear – a small set of factors can tell us, with significant accuracy, which cognitively normal individuals will develop mild cognitive impairment due to Alzheimer's," said Albert. "We hope that this information will be useful for designing clinical trials aimed at delaying the onset of symptoms among cognitively normal individuals. An approach such as ours could be used for determining which people might be most likely to benefit."

Saliva Test May Identify Normal Aging, MCI, and Alzheimer's – Early Results
Early detection of Alzheimer's related symptoms is critically important for individuals with the disease and for clinical studies seeking to slow or stop disease progression. However, many diagnosis techniques can be costly or invasive. Saliva is simple to obtain, easily transportable, and has been successfully used in a variety of diseases and conditions. Since multiple samples can be readily obtained, saliva testing is particularly useful for performing repeated assessments that span days, weeks, months or longer.

Knowing that Alzheimer's typically co-exists with certain metabolic disorders, Shraddha Sapkota, MSc, a neuroscience graduate student at the University of Alberta, Canada, and colleagues reported success at AAIC 2015 in identifying substances in saliva that differentiated among people with Alzheimer's disease (n=22), MCI (n=25) or normal aging (NA; n=35). A validation sample included 10 NA, 10 MCI, and 7 participants with Alzheimer's.

The researchers conducted their study using saliva samples, clinical diagnoses and cognitive data from the Victoria Longitudinal Study (VLS), a long-term, large-scale investigation of human aging. Protein analysis technology, called liquid chromatography-mass spectrometry (LCMS), was used to analyze the saliva samples and identify which substances were predominant in the saliva of each of the three types of individuals. Linking that data back to each participant's clinical diagnosis, researchers reported strong associations between certain substances and a person's cognitive abilities. For example, higher levels of one substance in the MCI group and another in the Alzheimer's group were observed. When these were examined in NA, higher levels of both predicted worse episodic memory performance. Another substance with higher levels in the Alzheimer's group predicted slower speed in processing information.

"Saliva is easily obtained, safe and affordable, and has promising potential for predicting and tracking cognitive decline, but we're in the very early stages of this work and much more research is needed," said Sapkota. "Equally important is the possibility of using saliva to find targets for treatment to address the metabolic component of Alzheimer's, which is still not well understood. This study brings us closer to solving that mystery."

Neurogranin, a CSF Biomarker for Synaptic Loss, Predicts Decline to Alzheimer's Dementia

Cerebrospinal fluid (CSF) buffers and protects the brain and spinal cord; when the brain is injured or damaged, certain proteins are released into the CSF. Examining CSF has led to the discovery of proteins that are strongly linked to Alzheimer's and can be useful in assessing an individual's health status. One such protein is neurogranin.

Neurogranin is a protein found only in the brain and is involved in the communication pathways between brain cells, known as synaptic signaling pathways. Synapse damage and loss is a common, early feature of Alzheimer's and there is a strong correlation between the extent of synapse loss and the severity of dementia. Recent research shows that neurogranin levels in the CSF are elevated in people with Alzheimer's.

At AAIC 2015, Maartje Kester, MD, PhD of VU University Medical Center, Amsterdam, Netherlands and colleagues presented findings from a study of 162 individuals from the Amsterdam Dementia Cohort who were either cognitively normal, had MCI or Alzheimer's. All of the participants had two cerebral spinal fluid samples taken over the course of two years, allowing researchers to compare the protein content over time. Participants also had cognitive exams about four years apart, giving researchers a picture of how participants' memory and thinking status changed over time.

The research team found that baseline levels of neurogranin were significantly higher in individuals with Alzheimer's than in cognitively normal individuals. Initial neurogranin levels were also significantly higher in MCI individuals who progressed to Alzheimer's compared to those with stable MCI, and they were predictive of progression from MCI to Alzheimer's. They found that neurogranin levels were strongly correlated with two other Alzheimer's-related proteins, tau and ptau-181, in all three groups.

The researchers also observed that while neurogranin increased slightly over time in the cognitively normal group, it did not in those with MCI or Alzheimer's. "This may indicate that neurogranin levels in CSF reflect very early synaptic loss in Alzheimer's and may be useful for early detection," Kester said.

"We found that neurogranin is a potentially useful marker for the diagnosis, prognosis and monitoring of Alzheimer's," said Kester.

Imaging Inflammation in the Brain – Is It In Our Future?

PET imaging compounds for amyloid plaques in the brain have been FDA approved for use in people with suspected Alzheimer's disease but an unclear diagnosis or an unusual presentation, and, as reported at AAIC 2014, significant advances are being made in PET imaging of tau tangles. These are the two hallmark brain lesions of Alzheimer's disease and are thought to be involved in its cause and progression.

Inflammation is another condition/pathway being investigated for its role in Alzheimer's; it can be deadly to brain cells and may be activated by the plaques and tangles. Microglial cells, as the brain's immune cells, constitute the active immune defense in the central nervous system (CNS). They have the potential to either protect or – when activated – destroy critical links in the brain. Attempts have been made to develop treatments that keep the cells in a protective state.

Andreas H. Jacobs, MD, Professor at the European Institute for Molecular Imaging, Muenster, Germany, and Director of the Department of Geriatrics at the Johanniter Hospital, Bonn, Germany, presenting at AAIC 2015 on behalf of his collaborators from the INMIND consortium, gave an overview of the state-of-the-art in PET imaging of inflammation in the brain.

To monitor the effects of new treatments on microglial cells, researchers often use positron emission tomography (PET) scans to visualize active microglial cells by tracking a protein that is produced at higher levels when the cells are active/destructive. Use of this technique is limited, however, because the relationship between this protein and microglial function is not fully understood yet, making interpretation of imaging results challenging. Consequently, other imaging targets are now under investigation.

"The data reported at AAIC demonstrates the potential of new target structures – such as cannabinoid type 2, P2X7 and COX2 receptors – to more accurately reflect various stages or types of the microglial cells, telling us if they're being destructive or protective," said Jacobs. "It is our hope that these new imaging tools can help us assess the effectiveness of treatments that lessen their destructive behavior."

About AAIC
The Alzheimer's Association International Conference (AAIC) is the world's largest gathering of researchers from around the world focused on Alzheimer's and other dementias. As a part of the Alzheimer's Association's research program, AAIC serves as a catalyst for generating new knowledge about dementia and fostering a vital, collegial research community.
AAIC 2015 home page: www.alz.org/aaic/
AAIC 2015 newsroom: www.alz.org/aaic/press.asp

About the Alzheimer's Association[®]
The Alzheimer's Association is the leading voluntary health organization in Alzheimer's care, support and research. Our mission is to eliminate Alzheimer's disease through the advancement of research, to provide and enhance care and support for all affected, and to reduce the risk of dementia through the promotion of brain health. Our vision is a world without Alzheimer's. Visit alz.org or call 800.272.3900.

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- Marilyn Albert, PhD, et al. Using Combinations of Variables to Identify Individuals with Preclinical AD. (Funders: U.S. National Institute on Aging)
- Shraddha Sapkota, MSc, et al. Metabolomics Analyses of Salivary Samples Discriminate Normal Aging, Mild Cognitive Impairment, and Alzheimer's Disease Groups and Produce Biomarkers Predictive of Neurocognitive Performance. (Funders: Canadian Institutes of Health Research, U.S. National Institutes of Health)
- Maartje Kester, MD, PhD, et al. Neurogranin, a CSF Biomarker for Synaptic Loss, Predicts Decline to Dementia Due to Alzheimer's Disease. (Funders: Alzheimer's Nederland, U.S. National Institute on Aging)
- Andreas Jacobs, MD, et al. Imaging Neuroinflammation in Neurodegenerative Diseases (INMIND) – Concepts and Future Directions. (Funders: European Commission Directorate-General for Research and Innovation, European Union's Seventh Framework Programme for Research and Technological Development)

Proposal ID: F1-03-02
Featured Research Session, Sunday, July 19, 8-9:30 am, Ballroom C
Theme Selection: Diagnosis and Prognosis

Using Combinations of Variables to Identify Individuals with Preclinical AD
- **FRS Topic: Longitudinal Study of Pre-Symptomatic Alzheimer's Disease Biomarkers: A Foundation for Research on Prevention.**

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Background: Several lines of evidence indicate that a range of measures obtained in cognitively normal individuals are significantly related to time to onset of clinical symptoms of Alzheimer's disease (AD) that develop many years later. This suggests that combinations of biomarkers might be useful for selecting individuals to include in clinical trials and for assessing disease progression in response to treatment.

Methods: Using data from the BIOCARD study, which includes serial assessment of CSF, MRI and cognitive measures, we used time-dependent ROC methods to examine combinations of measures that were significant predictors of which cognitively normal individuals had a high likelihood of progressing to mild cognitive impairment (MCI) due to AD over time. The best model was selected using the AIC criterion.

Results: Using data from baseline in 189 participants, and predicting onset of clinical symptoms up to 5 years later, sets of biomarkers were examined using several approaches: (1) the least costly to the most costly, (2) the least invasive to the most invasive, and (3) the best fit model. The best fit model included 6 variables: two cognitive measures (Digit Symbol and Paired Associates Immediate Recall), two CSF measures (Abeta and ptau) and two MRI measures (right entorhinal cortex thickness and right hippocampal volume), adjusted for demographics (AUC=0.886, sensitivity = 0.85, specificity = 0.70). Moreover, the addition of each domain (cognitive + CSF + MRI) added significantly to the accuracy of prediction.

Conclusions: Combinations of biomarkers obtained at least 5 years prior to symptom onset can be used to identify which cognitively normal individuals will progress to MCI due to AD. Additionally, these findings demonstrate that biomarkers useful for prediction later in the disease course are useful in preclinical AD. Clinical trials aimed at delaying the onset of symptoms could utilize such an approach for determining which individuals might be most likely to benefit from inclusion in a clinical trial aimed at individuals with preclinical AD and/or tracking response to treatment.

Proposal ID: P3-090
Poster, Thursday, July 21
Theme Selection: Diagnosis and Prognosis

Metabolomics Analyses of Salivary Samples Discriminate Normal Aging, Mild Cognitive Impairment, and Alzheimer's Disease Groups and Produce Biomarkers Predictive of Neurocognitive Performance

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Background: Metabolomics is a global approach to detecting perturbations in metabolic pathways that can reflect early and subtle disease-related changes in the central nervous system. The metabolome, the end product of gene-environment interactions, can characterize and discriminate metabolic signatures of Alzheimer's disease (AD) and Mild Cognitive Impairment (MCI). We previously established the technology for using salivary samples for discriminating normal cognitive aging (NA) from MCI groups. We report pairwise metabolomics comparisons among new NA, MCI, and AD groups. Three main goals were to (1) fully discriminate the groups based on metabolomics signatures, (2) identify top discriminative metabolites, and (3) test these metabolites as biomarkers predictive of neurocognitive performance.

Methods: Salivary specimens, validated clinical classifications, and cognitive data were collected on two sets of three groups from the Victoria Longitudinal Study. The discovery sample (64% female) included NA (n=35; M age=69.94 years), MCI (n=25; M age=70.40 years), and AD (n=22; M age=77.09 years), whereas the validation sample included 10 (NA), 10 (MCI), and 7 (AD) participants (N=109). We used the established 13C/12C isotope dansylation labeling technique and liquid chromatography-mass spectrometry to identify metabolite biomarkers. The top pool of discriminant metabolites were identified using orthogonal partial least squares discriminant analyses (OPLS-DA) and Variable Importance in Projection indices (>1.5), Fold Change (>2.0), and goodness of fit (R2Y (cum)>0.9). Regression analyses (Mplus) determined biomarker-cognition associations for the top discriminative metabolites.

Results: First, M=1515 metabolites was detected in each pairwise comparison. Second, OPLS-DA revealed clear discrimination between all groups, and volcano plots identified top discriminant metabolites between each pairs. Third, a pattern of predictably directional metabolite-cognition associations were identified. For example, one metabolite was upregulated in the MCI group (log2 (FC)=1.11;AUC=0.732) and another metabolite (Trp Glu;Glu Trp) (log2(FC)=1.87;AUC=0.866) was upregulated in AD and, when examined as biomarkers in NA, higher levels of both predicted worse episodic memory performance. Another metabolite (Tyr-Asn-Ser) (log2(FC)=1.24;AUC=0.826) was upregulated in AD and higher levels in predicted slower neurocognitive speed in the AD group.

Conclusions: Metabolomics analyses can produce clinical discrimination, biological pathway information, and predict biomarker-cognition associations. Saliva is a non-invasive biofluid with promising biomarker translation implications in Alzheimer's research.

Proposal ID: O5-05-03
Oral Session, Thursday, July 23, 11:30 am–1 pm, #207
Theme Selection: Diagnosis and Prognosis

Neurogranin, a CSF Biomarker for Synaptic Loss, Predicts Decline to Dementia Due to Alzheimer's Disease

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Background: We examined the utility of a novel cerebrospinal fluid (CSF) biomarker of synaptic loss, neurogranin, for diagnosis, prognosis, and longitudinal monitoring of Alzheimer's disease (AD).

Methods: CSF levels of neurogranin were measured in 37 cognitively normal (64(2)y, f38%, MMSE 28(0.3)), 61 subjects with mild cognitive impairment (MCI; 68(1)y, f38%, MMSE 27(0.3)), and 65 AD-patients (65(1)y, f45%, MMSE 22(0.7)) from the Amsterdam Dementia Cohort who underwent two lumbar punctures (mean (SE) interval of 2.0 (0.1) years). Mean (SE) duration of cognitive follow-up was 3.8 (0.2) years. We used ANOVA (log-transformed CSF biomarker levels) to assess baseline differences, and Cox-regression (CSF biomarker levels in tertiles) to predict progression to AD in MCI. Linear Mixed Models were used to assess within-person annual change in neurogranin levels. All analyses were adjusted for sex and age.

Results: Baseline levels of neurogranin in AD were higher than in cognitively normal individuals (median (IQR) 2381 (1651-3416)vs. 1712 (1206-2724), p=0.04). Baseline neurogranin levels were strongly correlated with tau and ptau–181 in all clinical groups (all: Spearman r>0.77, p<0.001), but not to Aβ42. Baseline neurogranin levels were also higher in MCI individuals who progressed to AD compared to those with stable MCI (median (IQR) 2842 (1882-3950) vs. 1752 (1024-2438), p=0.004), and they were predictive of progression from MCI to AD (HR [95% CI] 1.8 [1.1-2.9]). Linear Mixed Model analyses demonstrated that within-person levels of neurogranin increased in the cognitively normal group (mean (SE) 90 (45) pg/mL per year, p<0.05), but not in those with MCI or AD.

Conclusions: Neurogranin is a promising biomarker for AD, as levels were elevated in AD compared to cognitively normal individuals and predicted progression from MCI to AD. Within-person increases in neurogranin in cognitively normal individuals, but not those with later stage MCI or AD, suggest that neurogranin reflects early synaptic dysfunction or loss.

Proposal ID: F1-02-04
Featured Research Session, Sunday, July 19, 8-9:30 am, Ballroom A
Theme Selection: Basic and Translational Science

Imaging Neuroinflammation in Neurodegenerative Diseases (INMIND) – Concepts and Future Directions
- **FRS Topic: Innate Immunity and Alzheimer's Disease**

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Background: Positron Emission Tomography (PET) is one of the key molecular imaging technologies which allows the location and quantification of the expression of molecular target structures in vivo by employing specific radiopharmaceuticals. PET allows the translational and reverse-translational assessment of disease-specific molecular alterations over time, during the disease course and under therapeutic intervention. The aim of the lecture is to demonstrate (i) the current state-of-the-art of tracer technology for assessing microglial activation and (ii) implementation of old and new microglial imaging strategies in various disease models and human application.

Methods: For 30 years, imaging the activation of microglia cells in vivoby PET is accomplished by employing the mitochondrial translocator protein 18kDa (TSPO) as molecular target. TSPO is over-expressed by activated microglial cells, and in some conditions also by astrocytes. Over the last decade, about 50 radiopharmaceuticals targeting TSPO have been developed and investigated, only some of them have reached clinical application so far. As the biology of TSPO in relation to microglial function is not fully understood yet, interpretation of TSPO-based imaging results is sometimes difficult. Therefore, new microglial target structures such as cannabinoid type 2, P2X7, and COX2 receptors, respectively, are all being explored for imaging.

Results: All research data presented originate from joint efforts within the INMIND consortium.

Conclusions: The ultimate goal is to differentiate various microglial phenotypes (such as M1 and M2) during the disease course for the overall aim to establish imaging-guided therapeutic modification of microglia function to enhance their neuroprotective and repress their neurodestructive function.

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