Current Methods and Future Research in the Diagnosis of Alzheimer's Disease

Gaganpreet Jhajj[1 2 3] * and Mark Farinas[4]

[1] Department of Chemistry, University of Alberta, Edmonton AB T6G 2G2, Canada
[2] Neuroscience and Mental Health Institute, University of Alberta, Edmonton AB T6G 2E1, Canada
[3] Department of Biochemistry, University of Alberta, Edmonton AB T6G 2R3, Canada
[4] Department of Medical Microbiology & Immunology, University of Alberta, Edmonton, AB T6G 2E1, Canada

Abstract — **The ability to detect the presence of many neurodegenerative diseases during the early stages has been done with limited success. This article will briefly explore biochemical characteristics of Alzheimer's Disease (AD), and current methods for detecting AD. These methods will be evaluated against how accurate and invasive these tests are as well as the time required to conduct one of these tests. As well the innovations made for detecting other neurodegenerative diseases and how these methods could be applied for detecting AD in the early stages. How a diagnostic test based off discussed detection principles will also be detailed in addition to the theoretical creation of a fluorescent assay that could be used as a detection method for AD.**

I. INTRODUCTION

Alois Alzheimer first diagnosed what would later become Alzheimer's Disease (AD) in 1908 (Alzheimer et al., 1991). Alzheimer's first patient stated that she felt "that she had lost [herself]" and claimed that she was possessed by demons (Alzheimer et al., 1991). Alzheimer, not willing to accept this, believed that his patient was suffering from a form of dementia. The disease was discovered after an autopsy was conducted on the patient, after which Alzheimer saw the physical renditions of AD. AD is primarily characterized through groups of matter glommed together that Alois Alzheimer identified as plaques and tangles. Since the discovery of the disease, new methods of diagnosing AD have been developed, but each with varying degrees of success. The more invasive direct methods of detecting AD have shown to have a greater accuracy; however, diagnosing the presence of a specific neurodegenerative disease post-mortem provides no tangible benefit to the patient and others suffering from AD. In this area of research, there is a great benefit in creating earlier methods of detection for AD as well as methods that are less invasive. The disease that was discovered over a century ago still eludes physicians and researchers in how to diagnose and treat this condition. This report will focus on evaluating current methods in the diagnosis of AD and future tests that could indicate the disease. These tests will range from physiological exams and imaging methods such as MRI which are less invasive, to more invasive methods such as obtaining cerebrospinal fluid for further analysis.

The type of AD that will be focused on throughout this report involves late-onset AD. There have been genetic populations which have shown to have a large presence of individuals with AD in very localized geographic regions (Sherrington et al., 1995). The mechanisms by which AD manifests itself in late and early onset AD differ widely. Often in early-onset populations, there is a large accumulation of plaques and tangles in the individual as early as 20 years old (Sherrington et al., 1995). Comparatively, mechanisms by which late-onset AD, and the many factors that go into the propagation of it, shows manifestation around the age of 65. Generally, individuals with early-onset AD have shown to have proportionally smaller brains than individuals who experience late-onset AD (Sherrington et al., 1995). Additionally, determining the difference between Alzheimer's and other types of dementia is important in determining how to better diagnose AD. Dementia is a more general term used to describe symptoms that impact memory and recall as well as a decrease in the performance of everyday activities, while AD is a specific form of dementia which has shown to get significantly worse over time and affects memory, language, and thought. Neither Alzheimer's nor dementia are considered to be a normal part of the aging process.

II. BIOCHEMISTRY OF AD

Plaques and tangles in AD are created by amyloid-beta and tau aggregates, respectively (Ballatore et al., 2007; Hardy et al., 1992). Beta-amyloid results from the amyloid precursor protein when it undergoes fragmentation (Hardy et al., 1992). The presence of amyloid beta protein fragments in a healthy brain is relatively low as these fragments are broken down and removed. When large amounts of protein fragments exist in the brain for prolonged periods of time, they can clump and form plaques (Hardy et al., 1992). These plaques can block signaling between cells and are known to cause immune responses that can result in the destruction of nerve cells (Hardy et al., 1992). Tangles are the result of twisted fibers inside of brain cells due to a protein called tau (Ballatore et al., 2007). In normal brains, tau forms the structure of the microtubule which allows for the transport of important substances such as food from one nerve cell to another (Ballatore et al., 2007). In AD, tau begins to aggregate, causing the collapse of the microtubule structure (Ballatore et al., 2007). This leads to twisting which ultimately leads to a disruption of transportation in the cell, resulting in cell death (Ballatore et al., 2007). The production of amyloid beta and tau aggregates have been seen to present in large quantities many years before the diagnosis of AD (Ballatore et al., 2007; Hardy et al., 1992). Usually, tau tangles and amyloid beta plaques begin production in the hippocampus which is responsible for short-term memory, implicating short-term memory loss as the first most immediate sign of AD (Hyman et al., 1984; Ballatore et al.,

2007; Hardy et al., 1992). From this point, the plaques and tangles invade other areas of the brain, causing a wide host of other symptoms.

III. COGNITIVE EXAMS

Physiological exams are diagnostic in the late stages of AD and dementia. By this point, the patient often has little chance to deal with the buildup of plaques and tangles and the long-term damage that has already been caused to various parts of the brain. Modern physiological examinations may involve simple recall tasks that are tracked over a large period of time to aid in determining the progression of the disease. As the disease progresses through different parts of the brain, individuals can see a loss in speech production, motor skills, and changing temperaments. AD eventually leads to the degeneration of key parts of the brain that control basic functions such as heart rate. Diagnosis of AD is often accompanied by individuals forgetting both short and long-term memories which is diagnostic as different memories are stored in different regions of the brain and this can determine how far AD has progressed. In a clinical and familial setting, patients who stop recognizing close relatives and friend were shown to be deep into the progression of AD. One of these tasks involves drawing a clock and filling in the numbers. The clock drawing task shown in Figure 1 is part of a larger assessment called the Montreal Cognitive Assessment (MoCA) (Price et al., 2011).

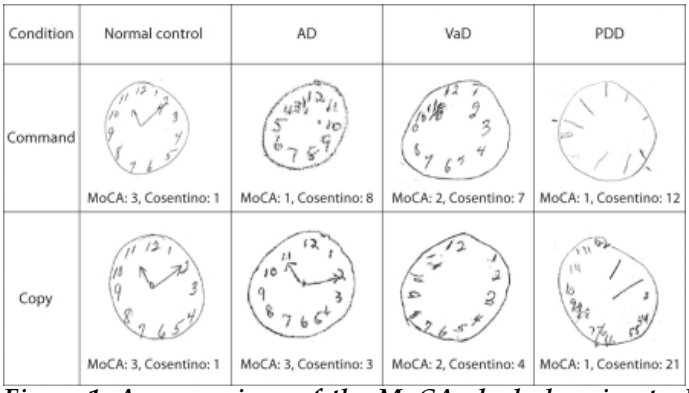

Figure 1. A comparison of the MoCA clock drawing task on individuals with AD, VaD (Vascular Dementia), and PDD (Parkinson's Disease Dementia). *Figure adapted from Price et al., 2011.*

This test consists of six categories, the first of which is a recall task, and the second of which is a task involving drawing a clock and a specific time, as well as drawing a three-dimensional cube (Price et al., 2011). Combined, the several cognitive domains that are tested on the MoCA create a scoring guide out of 30 in which an individual who has a score over 26 is considered to be normal (Freitas et al., 2013). Individuals with mild cognitive impairment scored an average of 22 and individuals with AD scored approximately 16 (Price et al., 2011). Various studies have affirmed the role of the MoCA as a diagnostic assessment for AD and MCI; however, it should be noted that there is detection primarily due to the late stage progression of AD in patients (Freitas et al., 2013; Price et al., 2011). As well, the MoCA being a specialized assessment looking for cognitive impairments, it is often not administered to patients who may have AD unless there is a strong indication that there is a presence of AD. This is usually the case when the disease has already progressed notably far that it is known there is some form of cognitive impairment. There is an immense need to diagnose AD earlier, to attempt to increase the quality of life for individuals who may be living with AD and increase their life spans.

IV. CURRENT LABORATORY TESTS

In a specific study using radiolabeled particles, researchers were able to determine the levels of tau and amyloid beta present in the brain through a variety of imaging techniques (Johnson et al., 2012). Imaging techniques have their own limitations in the diagnosis of AD, as it is often hard to distinguish specifically which neurodegenerative condition one might have merely through non-invasive imaging techniques such as MRI, CT, and PET scans (Johnson et al., 2012). Additionally, it is not possible for certain individuals to undergo certain methods of imaging which may be due to factors such as metal implants that make MRI not feasible and other psychological issues that may prevent someone from undergoing an MRI such as being uncomfortable with the loud humming of the electromagnet. There have been studies looking at the usage of a blood test for the detection of AD; however, this is not as indicative as using cerebrospinal fluid (CSF), a more valuable diagnostic indicator of AD (O'Bryant et al., 2016). The usage of a blood test however, does allow for positive indications of the potential presence of AD allowing patients to see more advanced diagnostic procedures such as CSF or further imaging (O'Bryant et al., 2016). Obtaining CSF for analysis for AD is one of the most invasive methods of diagnosing AD (Hansson et al., 2006). Getting access to CSF involves a complex procedure where many patients may not be willing to undergo as it can cause potential complications and discomfort. A more invasive method would be the use of a brain biopsy to diagnose AD. Biopsies come with their own associated risks and can cause unprecedented damage to the brain, resulting in why current methods in diagnosing AD are very limited in accuracy and early stage detections.

When evaluating the use of a diagnostic test, several key factors should be considered. Factors such as accuracy, cost, and time required to run a test should all be considered. The detail in the amount of information a test can provide should also be evaluated alongside of how invasive the test is. A matrix was constructed using this as a framework, and it is seen below in Figure 2.

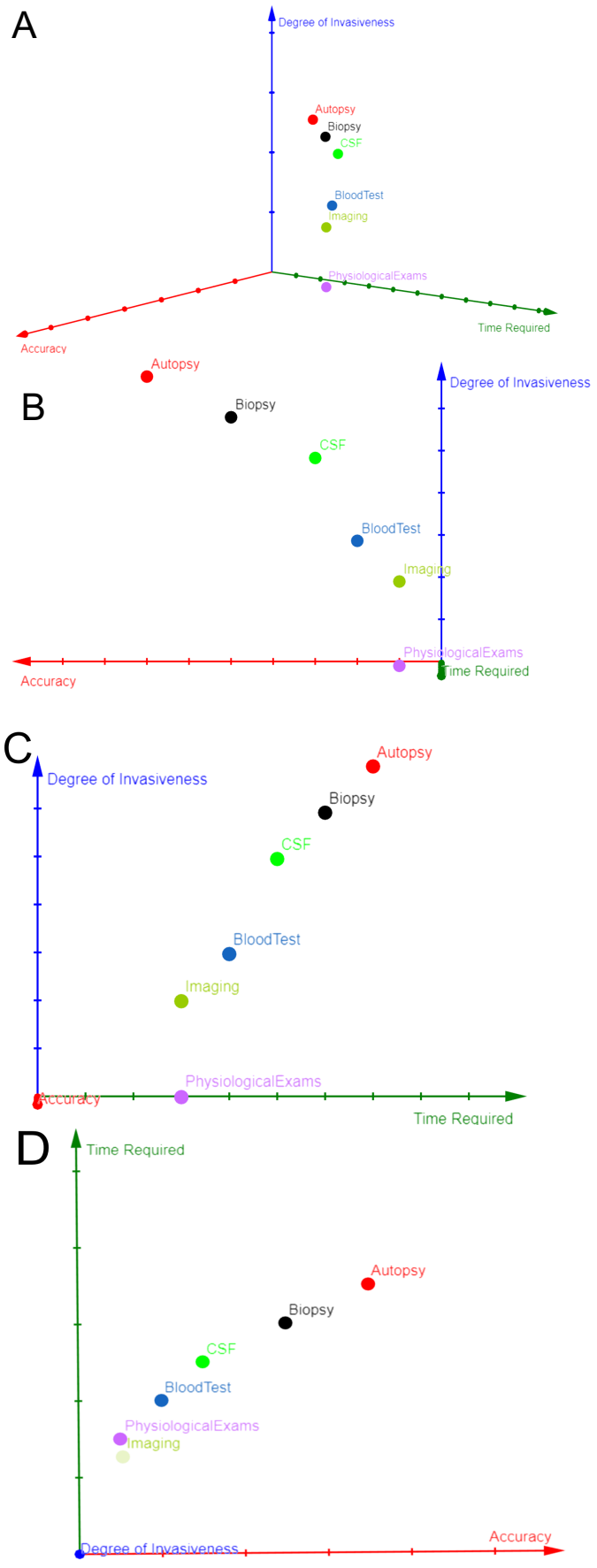

Figure 2. Comparison of six detection methods for late stage AD. *The effectiveness of six methods of detection for late-stage/end-stage AD were compared relatively using the factors of accuracy, invasiveness, and time required for tests. A Is the 3D overview of the graph while B to D shows in each 2D plane the relative performance of each method to one another. The values used for this graph quantitatively are arbitrary but provide a qualitative comparison between available methods. Image generated with (GeoGebra, 2018).*

V. CURRENT AND FUTURE RESEARCH

The current limitations in the detection of AD stem from the lack of accuracy in less invasive methods coupled with the fact that most of these methods are good at detecting late-stage AD. An ideal test would be able to detect precursors such as amyloid through an obtained sample such as blood. Thioflavin T (ThT) and Congo red can both interact and bind to fibrils which allow for the detection of the accumulation of amyloid. ThT has been shown to increase in fluorescence when binding to aggregated amyloid occurs. Using properties of diagnostic dyes and their derivatives, new tools and tests can be developed to detect the wide array of differently formed and shaped amyloid plaques. Applications of assays using ThT fluorescence to detect prion diseases has been shown to work in diseases such as Creutzfeldt Jakob disease (CJD) (Orrù et al., 2017). Using a process called real-time quaking-induced conversion (RT-QuIC) researchers were able to use CSF and nasal brushings to achieve a diagnostic sensitivity greater than 90% (Orrù et al., 2017). RT-QuIC was also used to diagnose Parkinson's disease (PD) through obtained CSF to quantify α-synuclein a major component of lewy bodies in PD (Groveman et al., 2018). A schematic of the process used in RT-QuIC can be seen below in Figure 3. This technology has the potential to be applied to the detection of AD.

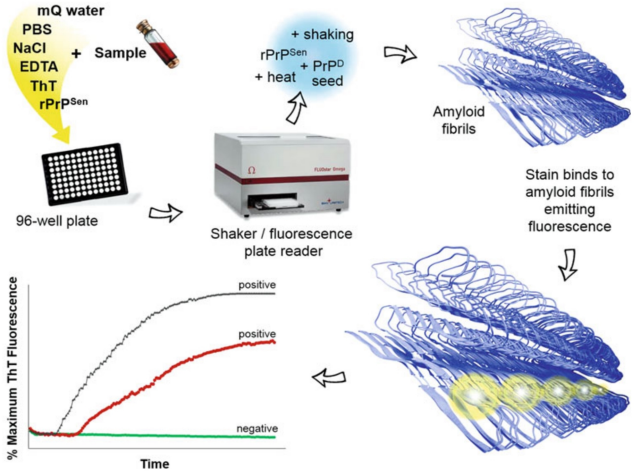

Figure 3. A schematic overview of the RT-QuIC detection method. *Figure adapted from Orrù et al., 2017.*

Using similar tests discussed in the previous literature for the detection of neurodegenerative conditions such as PD and CJD, there is future potential for the detection of plaques using ThT (Groveman et al., 2018; Orrù et al., 2017). A laboratory test could be developed in an attempt

to improve the detection of conformers of amyloid in obtained samples. This proposed method of detection aims to result in detecting AD during early stages of disease progression by identifying plaques while working towards using a less invasively obtained sample such as blood. Initially, an AD diagnostic test similar to that of CJD and PD would require the usage of CSF but the goal of this proposed bench test would be to improve the detection of AD in at least one of the domains of accuracy, time, or decrease invasiveness.

VI. DISCUSSION

Traditional methods of obtaining samples for amplification involve the use of harsh detergents which may result in loss of potential sample. Using seeding, the formation of amyloid fibrils can be accelerated (Groveman et al., 2018). Rapid techniques for the quantification of primary markers of AD is needed for a more rapid diagnosis. By using the cerebrospinal fluid (CSF) in new detection techniques, it is possible to identify and amplify specific biomarkers pertaining to a specific neurodegenerative condition. RT-QuIC has used α-synuclein (αsyn) seeds to provide a higher diagnostic accuracy when diagnosing PD (Groveman et al., 2018). The study used CSF from 29 synucleinopathy cases and 31 controls, which included 16 samples from patients with AD (Groveman et al., 2018). The diagnostic accuracy of this test was over 80% (Groveman et al., 2018). The CSF was obtained by carrying out a lumbar puncture, and approximately 20 mL of fluid was used for the RT-QuIC procedure (Groveman et al., 2018). αsyn seeds promoted amyloid fibril formation, which was then seen by the fluorescence of thioflavin T (ThT) (Groveman et al., 2018).

ThT is able to bind to amyloids at two sites with a high density (BS1 and BS2) and one at lower density (BS3). These binding sites are lined with hydrophobic and aromatic residues, as seen in Figure 4 (Reinke and Gestwicki, 2011).

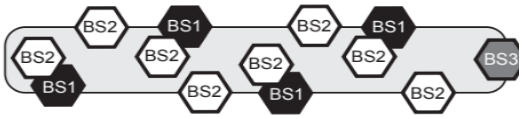

Figure 4. Representation of the different binding sites on the amyloid fibrils. *Figure obtained from Reinke and Gestwicki, 2011.*

When ThT binds to the β-amyloid fibrils, the fluorescence increases due to the change in carbon-carbon interactions between the aniline and the benzothiazole in ThT, as seen in Figure 5 (Reinke and Gestwicki, 2011).

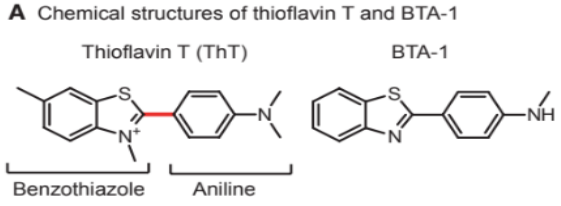

Figure 5. Chemical structures of ThT and BTA-1 to show the major system of a benzothiazole ring system attached to a functionalized aniline. *Figure obtained from Reinke and Gestwicki, 2011.*

When bound to fibrils, steric hindrance prevents rotation and allows the ThT to easily transition into an excited state (Reinke and Gestwicki, 2011).

Using an amine functionalized microplate, a Phosphate Buffered Saline (PBS) solution would be added and incubated on the microplate for a period of approximately 45 minutes (Interchim.fr, 2018). Afterward, the plate would be washed with the coupling buffer two times, and then the plate would be blocked with solution (Interchim.fr, 2018). The formation of amide bonds using 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) generates bonds between carboxylic acids and amines (Andersen and Denmark, 2018). EDC allows for the formation of a O-acylurea by activating the carboxylate. Using N-hydroxysulfosuccinimide (Sulfo-NHS), a hydrolysis resistant ester can be formed as under aqueous conditions the compound can undergo hydrolysis which greatly limits the obtained yield (Andersen and Denmark, 2018). The activated ester undergoes a reaction with Sulfo-NHS, which allows for the formation of a stable succinimidyl activated ester. This reaction can be seen in Figure 6 (Andersen and Denmark, 2018).

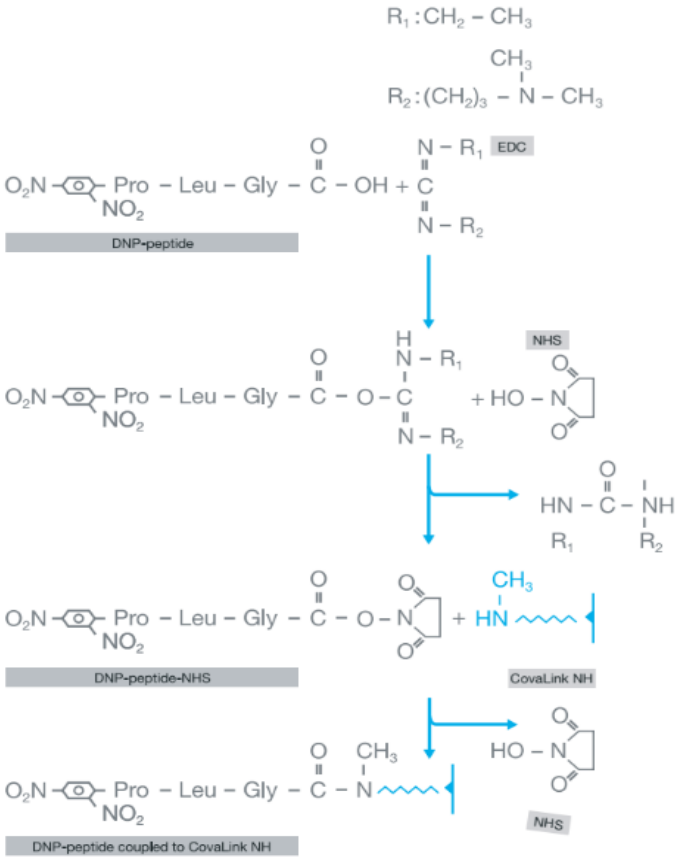

Figure 6. Reaction scheme for the immobilization of DNP-labelled tri-peptide. *Figure obtained from Andersen and Denmark, 2018.*

Using these, ThT cross-linked plates would allow for the capture of tau aggregates as well as amyloids and seeds in their native forms without degrading signal strength through the use of harsh detergents. Then, using recombinant monomers of amyloidogenic proteins, it would be possible to look for amplification. The amplification of certain amyloid proteins and a lack of others could aid in distinguishing between neurodegenerative diseases, if no cross seeding is present.

The study mentioned previously has had similar success when methods were applied to the detection of CJD (Groveman et al., 2018). The binding interactions between ThT and the tau fibrils also need to be understood to provide a more accurate baseline reading for the extent of aggregation present in the samples. The seeding assays initially will use samples that have come from patients confirmed of having AD and seeing how this method can amplify signals from a known source. As used in the method for both PD and CJD, CSF will ideally be used to run the RT-QuIC procedure. When compared to previous seed amplification methods, the RT-QuIC procedure allowed for more rapid quantification of αsyn seeds in the CSF (Orrù et al., 2017). This is all done by maintaining high degrees of sensitivity and specificity in diagnosing PD. The large success of this established protocol gives a promise for similar methods to be used for the detection of primary markers of AD (Groveman et al., 2018; Orrù et al., 2017).

Early stage detection of primary markers of Alzheimer's disease could provide immense health benefits for patients. In order for effective treatment of AD, methods such as the RT-QuIC protocol could be adapted and optimized for the quantification of tau seeds (Orrù et al., 2017). The CSF used in the RT-QuIC procedure resulted in a diagnostic accuracy greater than 80%, which is higher than conventional detection methods (Groveman et al., 2018). Investigating further the formation of fibrils in specific tauopathies would be needed to ensure this method has a greater accuracy. Effective methods to detect the formation of primary AD markers would allow for timely treatment to slow the progression of this disease.

VII. CONCLUSION

The early stage detection of Alzheimer's disease would provide the best health outcome for patients. The large populations in developing nations entail that there will be a rapidly increasing diagnosis of Alzheimer's in the coming years. The best way to decrease the burden on the health care system and allow patients to live normal lives for longer periods of time would be to slow the development of plaques and tangles. However, to provide correct treatment, there need to be effective early-stage diagnostic methods which currently there are a few that are proven to be reliable and non-invasive in nature. An ideal test would detect AD early, be minimally invasive, be accurate, and be able to be conducted rapidly. Innovations in any of these areas would greatly increase AD diagnosis and treatment. Innovations in the detection of CJD and PD could translate to future methods in detecting AD. Potential benchtop tests involving ThT and its binding interactions with amyloid could be developed in the near future using detection methods similar to the RT-QuIC detection method.

AUTHOR INFORMATION

Corresponding Author
Gaganpreet Jhajj* gjhajj@ualberta.ca

ABBREVIATIONS

Alzheimer's Disease (AD)
Thioflavin T (ThT)
Cerebrospinal fluid (CSF)
Creutzfeldt Jakob disease (CJD)
Real-time quaking-induced conversion (RT-QuIC)
Vascular Dementia (VaD)
Parkinson's Disease Dementia (PDD)

REFERENCES

Alzheimer, A., Förstl, H., and Levy, R. (1991). On certain peculiar diseases of old age. *History Of Psychiatry* 2, 71-73.

Ballatore, C., Lee, V., and Trojanowski, J. (2007). Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nature Reviews Neuroscience* 8, 663-672.

Freitas, S., Simões, M., Alves, L., and Santana, I. (2013). Montreal Cognitive Assessment. *Alzheimer Disease & Associated Disorders* 27, 37-43.

Groveman, B., Orrù, C., Hughson, A., Raymond, L., Zanusso, G., Ghetti, B., Campbell, K., Safar, J., Galasko, D., and Caughey, B. (2018). Rapid and ultra-sensitive quantitation of disease-associated α-synuclein seeds in brain and cerebrospinal fluid by αSyn RT-QuIC. *Acta Neuropathologica Communications* 6.

Hansson, O., Zetterberg, H., Buchhave, P., Londos, E., Blennow, K., and Minthon, L. (2006). Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *The Lancet Neurology* 5, 228-234.

Hardy, J., and Higgins, G. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256, 184-185.

Hyman, B., Van Hoesen, G., Damasio, A., and Barnes, C. (1984). Alzheimer's disease: cell-specific pathology isolates the hippocampal formation. *Science* 225, 1168-1170.

Johnson, K., Fox, N., Sperling, R., and Klunk, W. (2012). Brain Imaging in Alzheimer Disease. *Cold Spring Harbor Perspectives In Medicine* 2, a006213-a006213.

O'Bryant, S., Edwards, M., Johnson, L., Hall, J., Villarreal, A., Britton, G., Quiceno, M., Cullum, C., and Graff-Radford, N. (2016). A blood screening test for Alzheimer's disease. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring* 3, 83-90.

Orrù, C., Groveman, B., Hughson, A., Manca, M., Raymond, L., Raymond, G., Campbell, K., Anson, K., Kraus, A., and Caughey, B. (2017). RT-QuIC Assays for Prion Disease Detection and Diagnostics. *Prions* 185-203.

Price, C., Cunningham, H., Coronado, N., Freedland, A., Cosentino, S., Penney, D., Penisi, A., Bowers, D., Okun, M., and Libon, D. (2011). Clock Drawing in the Montreal Cognitive Assessment: Recommendations for Dementia Assessment. *Dementia And Geriatric Cognitive Disorders* 31, 179-187.

Reinke, A., and Gestwicki, J. (2011). Insight into Amyloid Structure Using Chemical Probes. *Chemical Biology & Drug Design* 77, 399-411.

Sherrington, R., Rogaev, E., Liang, Y., Rogaeva, E., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., and Holman, K. et al. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* 375, 754-760.