Pre-Clinical Alzheimer's Disease: A Survey of the Observed Working Memory Deficits Among Non-Demented APOe4 Carriers

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease that affects people of all ages but is most prevalent in the aging population, a population that is growing as the average lifespan increases. Patients with AD suffer extreme memory loss and eventually die. The disease causes severe cognitive decline, including a gradual decline in working memory (the ability to store, manipulate and recall information in a short time period). Treatment for AD is very limited in its effectiveness and the disease itself is irreversible. There is, however, a known genotype, apolipoprotein epsilon 4 (APOe4), which puts people at a higher risk for developing the disease in old age. It is also known that APOe4 carriers, before the onset of AD, perform worse on certain working memory tasks than education and age-matched non-carriers do. Working memory deficits have been observed on some tasks and not on others. Exploring the types of working memory tasks that differentiate the APOe4 carriers from the non-carriers under different conditions helps to further understand the cognitive changes that occur in pre-clinical AD and the link between the genotype and the disease.

Alzheimer's Disease

Alzheimer's disease is an irreversible neurodegenerative disease that mostly affects the elderly population. The average age of clinical diagnosis for AD is 65, and the average life span following diagnosis is 7 years (McKhann et al., 1984). Many elderly people suffer from dementia, which involves cognitive impairment and personality changes as a result of some form of disease or injury. AD is the most common cause of dementia among the elderly population. Estimates show that 5.1 million people in the United States are currently diagnosed with AD, over 1.5% of the United States population (McKhann et al., 1984). Approximately 26.6 million people have AD in the world today (World Population Prospects, 2006). With the baby boomer generation now in their 60s and the population at risk for AD growing, cases of AD are expected to nearly triple in the next forty years.

Late-onset AD is initially characterized by the inability to learn new skills, difficulty remembering how to navigate familiar places, difficulty multitasking, and mood swings (National Institute on Aging, 2012). As the disease progresses, patients experience more drastic memory loss and eventually forget their own identity, family, and friends. As the body weakens, the patient ultimately becomes unable to perform vital functions and the patient dies as a result of the disease (McKhann et al., 1984).

The neurological pathologies and symptoms of normal aging and AD are very similar, so it is often difficult to distinguish between the two. Brain scans and evidence from autopsies have provided two hypotheses for the unique neural pathology of AD--neuritic plaques and neurofibrillary tangles. Researchers have discovered abnormally high amounts of beta-amyloid deposits on neurons in the brains of AD patients (Kang et al., 1987). These deposits form plaques throughout the brain. These plaques also happen to be common in aging, but they occur more frequently in AD patients than in non-AD

patients of the same age (Kang et al., 1987). AD patients also have an abnormally high amount of tau protein in their brains, which causes neurofibrillary tangles to form in neurons (Perl, 2010). After time, the plaques halt new neuron growth and cause neuronal death (Lazarov & Marr, 2010). The progressive nature by which the plaques and tangles cause neurons to rapidly malfunction and die offers an explanation for how AD patients progressively lose cognitive abilities like the ability to acquire new memories. Brain scans also indicate that as AD patients age and the disease progresses, they have less grey matter than age-matched controls without AD. This difference in mass indicates that more neurons have died out in the brains of AD patients (National Institute on Aging, 2012).

Transgenic models of animals that are genetically altered to develop neural plaques and tangles provide strong support for the two hypotheses. The animals mature with an abnormally high amount of beta-amyloid in the brain, causing plaques and tangles to form. When given simple cognitive tasks the transgenic animals perform worse than control wild type animals (Oddo, Caccamo, Kitazawa, & Tseng, 2003). These models provide strong evidence that the plaques and tangles found in AD patients are responsible for the observed cognitive deficits (Oddo et al., 2003).

The Apolipoprotien e4 Genotype and it's Significance in AD

In 1993, Allen Roses discovered evidence that individuals who have the e4 allele on a gene responsible for encoding Apolipoprotein E (APOE) are more common among the AD population than the general population (Corder et al., 1993). Some studies indicate that up to 50% of people in the United States who suffer late-onset AD have the e4 allele (Raber, Huang, & Ashford, 2004). It should be noted that not all AD patients have the APOe4 genotype, and not all people with the APOe4 genotype develop AD. The likelihood of developing late-onset AD also increases with gene dosage. Homozygote APOe4 carriers, those with two alleles for the gene, are significantly more likely to develop AD than heterozygote carriers and non-carriers (Raber et al., 2004). There was evidence of a genetic link with AD Prior to the APOe4 genotype discovery, when it was found that the average person's chances of developing AD increased with familial history (Raber et al., 2004). The discovery of the APOe4 genotype thus provided further evidence for a genetic determinant of AD.

APO is an Apolipoprotein involved in the catabolism of a specific group of lipoproteins. It is expressed throughout the body, however it is most heavily expressed in liver cells and cells in the central nervous system. An APO allele occurs in nearly 80% of the population, but the e4 allele, which is less common, is the only variation that has any known correlation with AD (Raber et al., 2004). The percentage of people with the APOe4 allele differs between different cultures and is generally higher for Caucasians than most other cultures. Estimates suggest that somewhere between 20% and 30% of the population have at least one allele for APOe4 (Raber et al., 2004). This isoform has a greater difficulty breaking up plaques and tangles than other isoforms, so APOe4 carriers are more likely to have more plaques and tangles than average age-matched non-carriers (Pericak-Vance, 1991). Researchers concluded that this genotype thus makes individuals more susceptible to the plaques and tangles that cause AD (Pericak-Vance, 1991).

Working Memory

Memory is divided into two broad categories: long-term memory and short-term memory. Long-term memory is where people store information about their past, such as faces, places, and experiences. Anything stored in long-term memory was at one point retained in a person's short-term memory, where recent information is stored. A part of short-term memory that is closely studied by psychologists is working memory (WM), where people retain, manipulate, and recall limited amounts of information over a short time period (Becker & Morris, 1999). WM includes the processing of both verbal and non-verbal information.

Psychologists Alan D. Baddeley and Graham Hitch proposed a model of WM in 1974 where a central executive is responsible for moderating the information in two slave systems, the phonological loop and the visuospatial sketchpad (1974). The phonological loop continually rehearses a list of information for later recall. The amount of information that a person can hold in a loop ranges between 5-9 objects, differing between numbers and words (Miller, 1956). The visuospatial sketchpad is responsible for storing visual information, such as the color, shape, and location of objects. The central executive directs attention between the two systems and is responsible for blocking out irrelevant information (Baddeley & Hitch, 1974).

There are various types of tasks used to measure WM capacity. Researchers may present participants with images or words on a screen and test them later on the accuracy of their memorization, while also taking into account reaction time. Different methods of measuring WM often lead to different results for the same participant, indicating that WM has several components that can be highlighted with these various tasks. Unlike age-matched controls, AD patients suffer a progressive decline in WM. The severity of the WM deficits differs across WM tasks and at different stages of the disease (Baddeley, Bressi, Della Sala, Logie, & Spinnler, 1991). The age of onset differs per person and the rate of decline also differs between patients, so measuring WM in AD patients is challenging and often very inconsistent (Baddeley et al., 1991).

APOe4 Carriers and the Primacy Effect

In 2002, Rosen, Bergeson, Putnam, Harwell and Sunderland published the first paper that discussed the relationship between the WM of healthy APOe4 carriers and non-carriers (2002). They worked with a sample of 21 APOe4 carriers and 21 non-carriers. They did not report how many people in the sample were heterozygote or homozygote carriers, however since homozygote carriers are more rare it is likely that a majority of the participants in the carrier group were heterozygote carriers (Raber et al., 2004; Rosen et al., 2002). The participants in both groups ranged in age from 50-79 years old. All participants had no prior history of any neurological, psychiatric, or learning disorders, nor had they been prescribed any medication for pain or depression at the time of the study. None of the participants met the criteria for mild cognitive impairment, a test for dementia. The two groups were matched on age and years of education, with average age being approximately 62 years old, and the average years of education approximately 17 years. The two groups had similar IQs, according to the WAIS-R full-scale IQ (M=123 for carriers and M=125 for non-carriers). The two groups also did not vary from one another on the WMS-r general memory index (Rosen et al., 2002).

All participants were tested on the operation span task. In the task the participants were told to answer a series of math operations by confirming if the stated expression was true or false. For example, they would be presented with "8x2=16" and they would need to respond with either "yes" or "no". In between the math operations there would be short words that the participants were asked to remember for later recall. An experimenter monitored the task for every participant, so that the participant would not rehearse the words before recall (Rosen et al., 2002).

Rosen found that the math scores (percentage of equations answered correctly) and total span scores (total number of words recalled) did not differ significantly between the carrier and non-carrier groups. There was a significant difference ($p < .05$), however, between the carriers' and the non-carriers' primacy scores, a measure of how often the participant recalled the first word in the set. APOe4 carriers recalled the first word of the set on an average of 8.52 sets out of 15, compared to 10.43 for non-carriers (Rosen et al., 2002). The researchers then looked at the accuracy with which each group recalled the first word in the set, regardless of whether they remembered it as being the first word or not. These results were similar to the prior primacy results, implying that the WM deficits among APOe4 carriers were a result of poor word recall (Rosen et al., 2002). The operation span task forces participants to divide their attention between word recall and solving math operations. People with clinical AD show significant declines in divided attention, a central executive function (Baddeley et al., 1991). The results of the operation span task indicate that APOe4 carriers may experience similar types of WM decline as those seen among clinical AD patients (Baddeley et al., 1991).

Healthy APOe4 Carriers and Visuospatial Attention Tasks

In 2005, Greenwood, Lambert, Sunderland, and Parasuraman tested the second system of WM, the visuospatial sketchpad, within a sample of 113 non-carrier and 64 APOe4 carrier participants (12 of the APOe4 carriers were homozygous and 52 were heterozygous) (2005). The participants ranged in age from 41 to 85 years old, averaging 58 years old. Participants were excluded if they had reported having significant medical problems affecting their neuropsychology. The researchers also excluded all participants who did not fall within the normal age-related scores on the Mattis Dementia Rating and Wechsler Memory scales, as well as the Buschke Selective Reminding task. The researchers matched control non-carriers with two groups of carriers on age, intelligence and mental health (Greenwood et al., 2005).

Greenwood, Lambert, Sunderland and Parasuraman presented their participants with a task that measured spatial WM, the ability to recall locations within an image (2005). The participants were told to fixate on an picture on the computer screen at which point an "X" would appear for three seconds somewhere on the picture. After a delay, a red dot appeared on the picture, either in the same position as the "X" or in a novel position. The participants were asked to assess if the red dot location matched the target "X" location (Greenwood et al., 2005). The participants were asked to recall the location of one, two, or three target locations at a time. Under all conditions, the participants were asked to assess whether the location of the red dot was old or novel after viewing all of the target locations. Increasing memory load (i.e. asking participants to recall more target locations) increases task difficulty (Greenwood et al., 2005).

The results of the spatial WM task show a significant difference in scores between groups on the three locations condition, and little variation under the one and two locations conditions (Greenwood et al., 2005). Homozygote carriers, on average, performed worse than age-matched non-carriers and heterozygote carriers when asked to recall three locations. Homozygote carriers have a much higher risk of developing AD later in life than heterozygote carriers, so it was predicted that they would perform worse on cognitive tasks than heterozygote carriers (Greenwood et al., 2005). Patients with AD often discuss having difficulty remembering where they parked their car or how to navigate familiar areas, which both indicate a decline in visuospatial attention (National Institute on Aging, 2012). There is also evidence that AD patients perform worse than age-matched non-demented controls on certain visuospatial attention tasks, implying that the observed differences between the homozygote group and the other groups may be indicative of early signs of AD (Greenwood et al., 2005; Parasuraman, Greenwood, Haxby, & Grady, 1992).

Healthy APOe4 Carriers and the A-X Continuous Performance Task

Reinvang, Winjevoll, Rootwelt, and Espeseth ran a controlled study with APOe4 carriers using the A-X Continuous Performance Task (2010). The study included 186 participants who ranged in age from 40-80 years old and all participants were recruited through a newspaper advertisement in Oslo, Norway. All participants with a reported history of psychological disorders that affect cognitive abilities were excluded from the study. The experimenters also excluded all participants who scored more than one standard deviation away from the age-specific baseline average on the Wechsler Abbreviated Scale of Intelligence (Reinvang et al., 2010). The experimenters tested DNA from blood samples to assess genotype, and they observed that 69 of the 187 participants had at least one allele for APOe4 and 14 people were homozygote carriers (Reinvang et al., 2010).

During the task, participants observed a computer screen where red capital letters were continuously presented on a black background. The participants were told to respond with the rightmost key when presented with the target stimuli, the letter “A” followed by the letter “X”, and the leftmost key when presented with one of two non-target stimuli, the letter “A” followed by any letter but “X” (the AY condition), and any letter but “A” followed by “X” (the BX condition) (Reinvang et al., 2010). They were given 1500 ms to press the right or left key. The participants first did a practice block with 20 trials and then performed six blocks of 50 trials (Reinvang et al., 2010).

The researchers found that the male homozygote carriers (eight of the 14 homozygote carrier participants) performed significantly worse than all other participants in identifying the AY non-target condition. Homozygote male carriers achieved 63% accuracy, while all other groups achieved between 80% and 90% accuracy (Reinvang et al., 2010). Poor performance on the AY condition of the A-X CPT is indicative of poor response preparation. Changes that result from normal aging have been associated with poor performance on the A-X CPT (Emery, Myerson, & Hale, 2007). Since the neurological process of AD is a similar but much faster version of normal aging, these results serve to reinforce that association (Reinvang et al., 2010).

Healthy APOe4 Carriers Without Deficits in WM

The three previously discussed papers were all criticized for their sample sizes. The Greenwood et al. and Reinvang et al. studies included a sample size of nearly 200 participants, with around 35% of them carrying at least one allele for the genotype, and the Rosen et al. study included even fewer participants (n = 42) (Greenwood et al., 2005; Reinvang et al., 2010; Rosen et al., 2002). In 2007, Jorm and colleagues conducted a study comparing the cognitive performances of healthy APOe4 carriers to non-carriers with a much larger subject pool (Jorm et al., 2007). The Australian study recruited 6,500 Caucasian participants that were divided into three different age groups: 20-24, 40-44, and 60-64, with around 2,000 participants in each group (Jorm et al. 2007). The researchers recorded previous head traumas and surveyed their participants' levels of alcohol consumption, but did not eliminate any participants from the experiments based on the alcohol and head trauma data. They eliminated participants afterwards who were suspected of having dementia if they fell within a threshold on a dementia test, or if they had scores that were outliers (more than three standard deviations below the mean) on cognitive tests (Jorm et al., 2007). The experiment was conducted entirely on the computer and the participants were given the option of completing them at home or at the University with an interviewer (Jorm et al. 2007).

The experimenters used blood samples to test the genotype for all participants. 27.4% of the participants had at least one APOe4 allele, 2.3% had two alleles, and the results were consistent among age groups (Jorm et al., 2007). Under the category of WM test, the researchers tested all participants on a subset of the Wechsler Memory Scale, the backward digit span task (Wechsler, 1945). A series of digits appeared on a screen at one-second intervals and the participants were then prompted to recall the digits in backwards order (Jorm et al., 2007). The paper does not provide how many digits they were asked to recall.

The researchers observed no significant differences between heterozygote, homozygote, and non-carrier group scores on this task (Jorm et al., 2007). The researchers did, however, find differences between age groups, with the oldest group performing worse than the other age groups on all tasks, regardless of genotype. The researchers later looked at the effect of alcohol consumption and head trauma and found a correlation between the genotype and instances of head trauma and alcohol consumption. Using an ANOVA they saw that both head trauma and alcohol consumption had an effect on all participants' performance on cognitive tests, but the effect was not related to genotype. The tests were thus sensitive to age effects, head trauma, and alcohol consumption, but they were not sensitive to differences in people with the APOe4 genotype (Jorm et al., 2007).

Discussion and Further Directions

The Jorm et al. study presents an interesting counter-argument to the work of Rosen et al., Greenwood et al., and Reinvang et al.. Their results indicate that neither homozygote nor heterozygote APOe4 carriers exhibit abnormal cognitive decline prior to the onset of AD. Jorm et al. argue that based on the sample size, differences in WM scores between carriers and non-carriers in the other papers could easily be attributed to noise. The results from the Jorm et al. study are the most convincing, because their sample size was considerably larger than the others' (n = 6,500). With a much larger sample size, all of the studies that observed WM differences between healthy APOe4

carriers and non-carriers could guarantee with more confidence that their results were less influenced by noise. It is possible that the observed deficits in WM capacity among APOe4 carriers were too subtle to appear on Jorm et al.'s study. In their paper, Jorm et al. argue that, if their tests were not sensitive to APOe4 genotype effects, they would not have picked up age-related differences or differences associated with head trauma or alcohol consumption. Since there is no relation between AD symptoms and performance on the backward digit span task, it is not surprising that APOe4 carriers perform similarly to non-carrier controls. The task in question most likely does not measure a process of WM affected by AD or any of the neurological processes associated with AD. While no differences were observed between the carrier and non-carrier groups on any of the other cognitive tasks used by Jorm et al., such as an immediate and delayed recall, they are too broad to be considered WM tests (Jorm et al., 2007).

Rosen et al. provided strong evidence for a decline in primacy scores on operation span due to carrying the APOe4 gene. These results cannot be directly compared to Jorm et al.'s results, because number recall is different than word recall. Additionally, Jorm et al. did not report primacy scores for any of the tests, so a future study with a sample size as large as the one in question should examine primacy scores on an operation span task. Also, it is unknown how many people in the carrier groups were later diagnosed with AD. This knowledge would distinguish the effects of the genotype from the effects of early symptoms of AD (Rosen et al., 2002).

Greenwood explored age and genotype effects on spatial memory, and observed that homozygote carriers had a greater difficulty recalling a high load of target positions than heterozygote carriers and non-carriers. The Jorm et al. paper divided the heterozygotes from the homozygotes, but contrary to previous findings there were no observed differences between gene dosage on any of the cognitive tasks within each age group (Jorm et al., 2007). Reinvang et al.'s A-X CPT experiment also showed differences in gene dosage, but the A-X CPT measures a different resources of WM that cannot be easily related to any of Jorm et al.'s results, further limiting the relevance of Jorm et al.'s experiment.

The direct cause of AD remains unknown, but neuroscience research shows that plaques and tangles form in the brains of AD patients much more rapidly than in the adult aging brain. The discovery that healthy APOe4 carriers display deficits in WM prior to any symptoms of AD further emphasizes the link between the genotype and the disorder. As is the case with many diseases, understanding the cause can take us one step closer to finding a cure. Perhaps by using information learned about APOe4 carriers before the onset of AD, researchers can better understand the progression of the disease and potentially trace the decline in cognitive functioning before the onset of clinical AD.

Future studies should aim to replicate the previously discussed results using the WM tasks that have distinguished healthy APOe4 carriers from education and age-matched non-carriers. These experiments need to have a larger sample size, much closer to the Jorm et al. 2007 sample size ($n>6,000$) (Jorm et al., 2007). Researchers should also consider combining WM tasks and other measures of fluid intelligence to create a composite score that serves as a latent variable of WM. Additionally, researchers should return to the data several years later and compare the performance of the APOe4 carriers who later develop AD to those who do not. The scores of the non-carriers who later develop AD should be compared to the non-carriers who do not develop AD. Being able

to control for later development of AD will help distinguish whether the observed differences in WM are solely related to the genotype or are simply early symptoms of AD, too subtle to show up on tests that measure dementia.

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