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Alzheimer's Disease: Current State of Molecular Genetics Research and Treatments

Introduction

Alzheimer's disease (AD) is a progressive, degenerative neurological disorder resulting in dementia, loss of memory, change in personality and loss of cognitive functioning with its prevalence increasing with age. Approximately 4 million Americans have AD, with 22 million individuals worldwide having AD by 2025. AD costs the U.S. more than \$100 billion a year, with private health insurance failing to cover most long-term care needs of patients. As the population lives longer due to lifestyle changes and health technologies, research into the molecular genetics of AD will become increasingly important to accommodate the shifting demographic. The aim of this paper is to provide a background on AD research, focus on key chromosomal AD determinants, review current state of AD management, and propose future directions for study and treatment.

Milestone Research Findings

There was little known about the molecular genetics of Alzheimer's disease until about 20 years ago. German physician, Alois Alzheimer, named the characteristic symptoms of AD in 1907. An autopsy of a patient with the degenerative neurological symptoms revealed dense deposits of plaques, and tangled strands of fiber in and around the nerve cells of the brain. Zimmerman conducted further studies in 1962 into the

topographic neurofibrillary changes of an AD brain and the fine structure of the neurofibrillary tangles was first elucidated by electron microscopy in 1963.

Studies by Whitehouse (1) in 1981 provided evidence for the selective loss of central cholinergic neurons in the nucleus basalis of Meynert. A decrease in neurons of greater than 75 percent and selective degeneration was seen in patients with AD.

More insight was gained into the late-onset nature of AD by a series of reviews by Bartus (2) in 1982, which discussed the role of cholinergic dysfunctions in age-related memory disturbances. Significant cholinergic dysfunctions were found to occur in the aged and demented central nervous system, and a strong relationship was found between the physiological changes and loss of memory. Studies were able to induce similar memory deficits by artificially blocking cholinergic mechanisms in young subjects, and memory improvements were achieved after cholinergic stimulation. These studies into natural age-related memory disturbances of geriatrics pointed to pronounced cholinergic dysfunction as a significant cause of memory loss in late-onset AD.

Extensive work was conducted in the early 80's to investigate the nature of the amyloid plaques that are present in an AD type brain. Merz (3) studied the ultrastructural morphology of amyloid fibrils from neuritic and amyloid plaques, while Glenner (4) reported on the purification and characterization of an amyloid protein derived from a serum precursor of twisted beta-pleated fibrils in AD patients. The serum precursor was thought to provide a potential diagnostic test for AD.

In 1984, extensive examination of temporal lobe from AD patients revealed a specific cellular pattern of pathology in the subiculum of the hippocampus. The affected cells were those that interconnected the hippocampus with the structures crucial to memory

such as the association cortices, the basal forebrain, the thalamus and the hypothalamus. It was found that the cell-specific pathology of the hippocampus disrupted many of the input and output processes to and from the hippocampus, most likely contributing to the memory dysfunctions in AD patients.

In 1985, purification and structure elucidation techniques allowed researchers to characterize the cerebral amyloid protein that form the plaque core in AD. Interestingly, the same amyloid plaque core protein was found in aged individuals with Down syndrome. The amino acid composition, molecular mass, and NH₂-terminal sequence was determined and the amyloid plaque core protein was found to consist of multimeric aggregates of polypeptide of about 40 residues (4 kDa).

Tau protein became a major source of research starting in 1986 when their presence was associated with AD's neurofibrillary tangles and plaque neurites. Tau proteins are microtubule-associated proteins thought to regulate different properties of neuronal microtubules, including their stability and orientation. It was hypothesized that abnormal Tau protein synthesis led to the formation of intraneuronal deposits that are often seen in AD type cells.

Genetics and Alzheimer's disease

The past several years have yielded new developments in the research of Alzheimer's, a large part being in the area of genetics. Sequencing and cloning techniques have provided researchers with a clearer picture of the proteins altered in AD patients.

Chromosome 21

In February of 1987, a series of studies aimed at discovering a genetic basis for AD found that the gene encoding beta-amyloid peptide in cerebral vessels and neuritic plaque of people with early-onset familial AD and Down syndrome is located on chromosome 21. Using genetic linkage to DNA markers on chromosome 21, researchers were able to determine the chromosomal location of the defective gene. Overexpression of the amyloid beta protein gene in brain tissue of patients with Down syndrome (trisomy 21) further supported the hypothesis that the dosage of the beta protein is related to the pathology of AD and Down syndrome.

A similar study by Delabar and Goldgaber (6) in March of 1987 showed that patients with AD have a duplicated region of chromosome 21 containing the beta amyloid protein-coding gene. Later that year, researchers used the amino acid sequence of the amyloid peptide to synthesize oligonucleotide probes specific for the gene encoding this peptide. A clone was found with a 1.7-kilobase insert that contained a long open reading frame coding for 412 amino acid residues including the 28 amino acids of the amyloid peptide in a human brain cDNA library when screened with a probe. Homologous genes were found in the genomic DNA of humans, rabbits, sheep, hamsters and mice, suggesting that the gene had been conserved through mammalian evolution.

A *Nature* publication in 1991 released the findings of a study that segregated a missense mutation in the amyloid precursor protein gene with familial AD. Examination of markers along the long arm of chromosome 21 in a single family with AD confirmed by autopsy, revealed a point mutation in the amyloid precursor gene. The mutation causes an amino acid substitution close to the carboxy terminus of the beta-amyloid peptide.

Cloning and sequencing techniques helped identify a double mutation on the beta-amyloid precursor protein gene in 1992, in early-onset AD families. Two base pair conversions, G to T and A to C, from the normal sequence were showed to yield Lys to Asn and Met to Leu substitutions.

In November of 1992, researchers at MIT isolated a cDNA from a mouse brain library that encoded a protein whose amino acid sequence was 42% identical and 64% similar to that of the amyloid beta protein precursor. In the same month, a carboxyl-terminal 100-amino acid peptide of beta amyloid precursor protein was expressed in transgenic mice under the control of the brain dystrophin promoter. The development of the transgenic mice gave researchers a preliminary animal model in which to study the cause and effect of amyloid-like fibrils in vitro, and preclinical model for testing the safety and efficacy therapeutic drugs.

The following month, a direct link was established between familial AD genotype and the clinicopathological phenotype when cultured cells from a Swedish AD family bearing the double mutation for the beta-amyloid protein precursor gene produced approximately 6-8 fold more amyloid beta-protein than cells expressing the normal beta-amyloid precursor protein gene.

Chromosome 19

The 3 most commonly observed genetic alterations are seen in the Apolipoprotein E (ApoE) gene on chromosome 19. It is involved in late onset AD, which is the most frequent form. While a definite pattern of transmission has yet to be determined, family history of a relative with dementia seems to be the most important risk factor. Linkage

and segregation analysis have concluded that this form does not exhibit Mendelian transmission properties as this suggests that this form may include effects of other loci other than the ApoE.

An ApoE gene is by no means unique to AD patients. ApoE4 is involved in the transport of cholesterol in and out of cells, facilitating the removal of cholesterol from plasma. While ApoE2 is another version of this gene, ApoE3 is the most common in the general population. Everyone inherits 2 of these ApoE genes, one from each parent. The ApoE4 gene is seen in two-thirds of late-onset AD, but this is not limited to only people with a family history of the disease. Individuals having this gene are three times greater at risk than the general population. Interestingly, the ApoE2 allele appears to be a protective form of ApoE. It lowers the risk of disease and increases the age of onset.

Like the amyloid peptide gene on chromosome 21, the effect of the ApoE4 gene seems to be dose-related. The presence of a ApoE4 gene lowers the age of onset for AD symptoms, while having two copies of the gene increases the chances for developing symptoms below the age of 70 by eight times, compared with someone who has two copies of the ApoE3 gene. The average age of onset of AD for people with no copies of the ApoE4 gene is 85 years.

Although the ApoE gene is situated in all neurons of both healthy and AD patients, it is localized in the senile plaques and neurofibrillary tangles that are seen in the affected persons. The presence of the ApoE4 seemed to best predict whose condition would decline, as this gene appears to mark susceptibility to AD. However, having the gene does not mean one will get the disease, and one may get the disease without ever having the gene.

The ApoE4 gene produces a protein that binds tightly to beta-amyloid, the ApoE3 protein. Beta-amyloid is normally soluble, but the attachment of ApoE4 protein makes it insoluble and more likely to be deposited in the plaques.

Chromosome 1 & 14

In 1995, three new missense mutations, all associated with the early-onset familial AD, were found on chromosome 1. The amino acid sequence for gene was homologous to the recently identified and cloned chromosome 14 AD gene. The gene region on Chromosome 1, called presenilin II had been found to be common among AD affected people who descended from Germans who lived in Russia near the Volga River. This inherited form strikes between the ages of 50 and 70 and shows many similarities with chromosome 14. This is still considered another rare mutation.

Treatments

While no cure exists for Alzheimer's disease, the best form of management starts with early detection. Positron Emission Tomography (PET), a technique that assesses the cerebral metabolism of glucose, has proved helpful in detecting the decreased metabolism that is commonly associated with advancing AD and decreased cognition. A variety of approaches have been studied and tested to help alleviate the symptoms of AD after detection.

Cholinergic Pathways

Post mortem studies of AD brains have shown lowered levels of acetylcholine and degeneration of cholinergic neurons. Because cholinesterases are found in senile plaques and neurofibrillary tangles, current treatments now include drugs that block the action of these enzymes. AChE inhibitors, such as tacrine, donepezil, and rivastigmine have been used to increase cognitive function in AD patients. However, due to high risk factors, tacrine is not often prescribed.

Antioxidants

Amyloid beta peptides, a main constituent of the plaques found in AD patients, increase the activity of tissue damaging free radicals. The free radicals inhibit astrocyte glutamate transporters, limiting the uptake of glutamate and causing cell death.

Antioxidants such as Trolox aim to limit the effect of free radicals on cells.

Anti-inflammatories

T-lymphocytes infiltrate the lesioned tissue of AD patients leading to inflammation. Glucocorticoids and non-steroid anti-inflammatories are used to help the verbal, spatial recognition and orientation functions of patients with AD.

Estrogen

Estrogen use by postmenopausal women offers many health benefits in bone density and resistance to fractures, but recent studies by Jacobs and Tang (8) have also shown that estrogen has a role in delaying the onset of AD significantly. More studies are

needed to determine the dose and duration of estrogen required, and to assess its safety in elderly postmenopausal women.

Future Research and Conclusions

Great progress has been made in the understanding of the molecular genetics of Alzheimer's disease. Quicker and less-expensive detection techniques of Apolipoprotein E genes will allow treatments to be tailored to each individual, based on unique risk factors from familial history and genotype. Substances that mimic ApoE2's effect could be developed to help slow or prevent the progress of Alzheimer's disease. Improvement on current vaccines could help prevent and dissolve plaques in Alzheimer's diseased tissue. Hopefully with increased efforts and new approaches to the research of aging, degenerative diseases and dementia, Alzheimer's patients will soon be able to maintain a considerably more productive and enjoyable standard of living.

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