Alzheimer's disease Genetic Factors - Twin Concordance Study

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***Abstract*:** Alzheimer's disease (AD) is a neurodegenerative disease and the leading cause of global dementia. It affects people equally regardless of race or gender. The clustering of AD in a family has not gone unnoticed. Defective genes associated with AD have been found on chromosome 1, 14, 19, and 21. The defect in chromosome 1, 14, and 21 which is associated with Early Onset of AD (EOAD) is believed to be associated with Amyloid Precursor Protein (APP) gene. This protein is believed to be associated with neurofibrillary tangle that that is associated with dementia of Alzheimer's type. The defect found in chromosome 19 is believed to be associated with apolipoprotein E (APOE). The APOE gene that is associated with chromosome 19 is believed to be involved with the Late Onset AD (LOAD). Molecular genetics techniques such as Restricted Fragment Length Polymorphism (RFLP) and Polymerase Chain Reaction (PCR) were used to determine genetic loci on the chromosomes mentioned above. These instruments were also used in revealing the deoxyribonucleic acid (DNA) sequencing and the area of mutation that caused the base substation. An example of such substitution is valine to isoleucine. If AD is hereditary, identical twins should have the same disease. The studies performed by many researchers and investigators, noted that genetic even when not completely responsible for AD, play a key role in the disease outcome. This study revealed that monozygotic concordance of AD patients is about 50% while dizygotic concordance is around 8%. However, these percentages do not agree with Mendel's segregation pattern of inheritance thereby requiring further studies.

PURPOSE

The occurrence of Alzheimer's disease (AD) has not gone unnoticed. Over the past several decades, families have been seeking professional evaluation. The evaluation process should involve family members as a whole. The family members should be tested for abnormalities in chromosome 1, 14, 19 and 21 starting with the individual that has been diagnosed with AD. There are several technologies available today like PCR, Flow Cytometry and etc.

Genetic counseling of AD patients should proceed psychological evaluation before blood or body fluid are drawn for DNA testing. The result if positive should be followed by another psychological counseling. Also body fluid and blood of these families could be incubated, then harvested and the chromosomes analyzed for possible mutations on chromosomes 1, 14, 19 and 21 which are believed to be associated with AD. A control study is also essential in obtaining quality results.

The fact is that the exact cause of AD is not yet known and generally accepted cause for the disease has not yet been established. Control studies will contribute to a great variation in Chromosome analysis. Due to many factors that cause AD, the question that often arises is why certain classes of nerve cells are vulnerable and subject to cell death. Those nerve cells are characterized by lack of neurotransmitters (Chemicals that transmit nerve impulse). The purpose of this thesis is to determine if a genetic factor causes AD and if it does, who is at risk.

In order to achieve this purpose, genetic studies will be evaluated and limitations, pitfalls, or sources of error will be discussed. The case studies and others discussed in this text were used to establish the fact that genetics plays a key role in developing AD. Included in this thesis is discussion of risk factors for AD and future prospective for AD.

INTRODUCTION

Genetic factors as the cause of Alzheimer's disease (AD) in humans is important in determining individuals at risk. Alzheimer's disease is a progressive degenerative disease of the brain now considered a leading cause of dementia. It was first described by Alois Alzheimer's in 1906, whom the disease was later named. Alois was a German neuropathologist (1).

Several recent studies have yielded new evidence that suggests very strongly that genetics may be a powerful determinant to AD. Before going into the genetics of the disease, some understanding of the basic genetics (the science that deals with hereditary) frame work of humans is necessary. Genetics is

responsible for transmission of characteristics from parents to offspring which can be divided into two forms. I) The gene make-up of the characteristics is called GENOTYPE, 2) whereas the physical manifestation of those characteristics is called PHENOTYPE. Every individual has 46 chromosomes and among these 44 are autosome and the remaining 2 are sex-linked (X and Y) chromosomes. Of these chromosomes from 1 to 44 which is actually 22 pairs, a defect has been found in chromosomes #s 1, 14, 19 and 21 that is associated with AD.

This does not mean that the defect appeared on both pairs. Each of these chromosomes has genes that carry information encoded in for protein synthesis. If these genes have defects (wrong code and if dominant), they will in turn present wrong messages for synthesis of the proteins. Scientists believe these defective genes affect the productions of amyloid precausor protein (APP) and Apoliprotein E4 (APOE) that has been found to be associated with Alzheimer's disease (AD). (2) (4) (29) (23) (48)

The gene location as well as the abnormal protein that is responsible for producing the precursor protein has been identified. Alzheimer's disease is characterized by lack of neurotransmitters (the chemical that transmit nerve impulses). However, scientists have been baffled by the issue concerning Alzheimer's disease, why certain classes of nerve cells lack this chemical and are vulnerable and subject to cell death. This review is pursuing an answer to the question above by investigating the effects of genetic factors (1) (2).

In the last few years significant progress has been made in identifying and describing different gene manifestations. Gene sequencing and linkage analysis studies have produced evidence of a possible locus on chromosome 21 in a small group of families with early onset familial Alzheimer's disease (FAD); Alzheimer's patients who have at least one relative affected by the disease are categorized as "Familial". Familial does not necessarily mean genetic. It could be due to environmental exposure. If one person had AD and no other family members are known to be affected, they are said to have "Sporadic" AD. (1)

The disease also appeared to be caused by an unidentified gene on chromosome 14.
The epsilon 4 Allele of apolipoprotein E (APOE) gene, which has been mapped to chromosome 19, is associated with the likelihood of developing the disease. Scientists searching for a gene that is involved in late onset of AD two and half years ago, discovered the involvement of APOE gene. The E4 type of this gene is very common on all late onset cases but hardly found in people without the disease. This E4 type of APOE gene is now being considered as the risk factor for AD. Eventhough Alzheimer's disease is the disease of the elderly, there is some literatures that support the notion that segregation of an autosomal dominant gene (gene that is found in non sex chromosome with a dominant allele) plays a key role in early onset trait. However, late onset nature of the disease blurs such distinctions, making it impossible to accurately answer the question how much AD results from genetic factors. Some investigators using the molecular

genetics linkage techniques, during the past six years have identified specific regions of four different chromosomes namely 1, 14, 19 and 21 that play a role in some cases of the disease and not in others (2) (18) (19) (23)

As genetic findings continue, they provide a framework so that relatives of pedigree (line of ancestor or family tree) can seek appropriate clinical counseling. Because of growing evidence of genetic causes of AD, clinicians are often questioned in regards to the risk of AD among relatives of patients. As people advance in age; the more the risk factor of developing AD. Although AD can affect people between the ages 30 to 50, themajority of the victims are above the age of 65. The fact that you have family members that have developed AD does not mean you are going to come down with AD. The rate at which AD occurs is the same all over the world and therefore race has nothing to do with the risk AD. Also gender has no part in developing AD. People that have a family history of Down's syndrome and Parkinson's disease are at a much greater risk, according to the result of some studies. But more research is needed to be definite. Some people have good reason to worry about the future. Over the past 20 years, people have watchedhelplessly as AD claimed the lives of their relatives. The medical experts were telling people a variety of things from the theory that there might be more than on gene involvedto the theory of where the gene is located. The study of genetics of Alzheimer's disease are yielding fundamental leads to the family of AD patients.(19) (21) (23)

Are there indicators that will help determine whether a genetic factor is involved in an individual Alzheimer's patients? In most cases, it is currently impossible to say; however, several scientific clues uncovered during the past 5 years are pointing to a concrete place to look, like chromosomes 1, 14, 19 and 21. Scientists have long known that people with Down's syndrome develop brain changes by the time they reach the age of 40 that appear identical to those of AD. Armed with this knowledge, researchers began studying a wide range of other families with multiple members affected with AD, attempting to find out whether there is consistent change in a gene on chromosome 21 andits presence in people with the disease. The first actual gene mutation associated with ADwas identified in the APP gene on chromosome 21. This gene mutation was found in members of two unrelated families with early onset FAD. The mutation on chromosome 14 which is associated with early onset AD revealed an area of chromosome 14 called AD3 which was found to have a gene which was called Sl82. (88)

Recently, investigators have discovered a defective gene on chromosome 1 that is similar to the gene found on chromosome 14 named Sl 82. This gene on chromosome 1 was discovered in an individual of native Russian decent. It was called STM2 (second- seven trans-membrane gene associated with AD) based on the location. This gene might also be responsible for early onset AD if the protein that this gene encodes for is similar in function to that ofS182 of chromosome 14. (45)

It is only recently, that late onset has been associated with genetic factors. One variation of a gene on chromosome 19 is more common in patients with late onset FAD than it is in people without the

disease. This version of gene is called APOE-E4, is present in 15% of the general population but in 50% of the case of late onset FAD. The evidence of this gene points to the first biological risk factor for late onset AD, other than age.

Knowing that the presence of the risk factor means the person may develop the disease,the presence of mutation in the gene means that the persons chances of developing the disease has increased. (88)

I am doing this review because personally, I have a lady friend whose father has developed an AD and I am curious to know if my lady friend is going to come down with the disease, also the society as a whole needs to know if it is inherited because that will better prepare people for counseling. This review is going to suggest that relatives of AD patients are at increased risk for the disease. I am trying to identify if genetic factors influence risk as well as what can be done to reduce the risk. (23)

LITERATURE REVIEW

According to Ralph W. Richter and John P. Blass; Alzheimer's disease is reported to be the fourth leading cause of death in the United States, afflicting about 3% of the U.S. population or 10% of those above the age 65. There are three types of AD namely Sporadic AD, Familial AD and inherited AD. The cases of inherited AD have ranged between 10% to 100% showing; that more research is necessary in order to clarify the 10% to 100% statistical range. All forms of AD mentioned above, appear to have chromosomal abnormalities associated with them. Genetic Studies to date have shown defects on Chromosome 1, 14, 19 and 21. (83) (84)

FAMILY AND GENETIC CASE STUDIES

Alzheimer's disease affects people equally regardless of race or gender.

Remembering that humans and other animals have 22 pairs of chromosomes and (X and Y) chromosomes, depending on the gender; if one of the pairs of 22 chromosomal genesis defective while the corresponding pair is normal, the individual still has a chance of developing the disease as long as the defective gene has been inherited.(6) (47) (48) (50).

The clustering of AD in a family is fully recognized. In patients with AD, the discovering of the gene coding for amyloid processing protein (APP), the linkage of early onset to chromosome 14 and that of late onset to chromosome 19, strongly suggest that AD is genetically transmitted. The purpose of the family and genetic case studies was to clarify the genetic transmission of AD using population based studies for early onset. The study is intending to find out the risks of first degree relative (blood relation like parents, siblings and offspring) of early onset AD patients. Sjogren in 1952 conducted a study with 890 first degree relatives, 18 were dementia of Alzheimer type (DAT) and 44 were Alzheimer Syndrome proband (identified individuals diagnosed with the Alzheimer's disease). The study was to show how Alzheimer cases clustered within certain families.

He concluded that the disease was probably caused by multi-factorial inheritance. This study was done on AD patients with pre-senile type of syndrome at mean age of 54 years and not beyond the age of 70 years. The study was not conclusive. But on the study of 1,412 relatives of senile dementia by Larsson in (1963), with the mean age of onset of 74 years, and aged 65 and older, the author concluded that a single dominant gene is responsible forthe transmission. (6) (13) (14) (31) (93)

Another study that was done between 1980 and 1987 of patients that came from several homes including nursing homes, psychiatric homes, and so on. These patients were independently examined and diagnosed with probable AD. They used a standard protocol similar to National Institute of Neurological Communicative Disease and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria. The patients that were included have probable AD, other dementia like multi- infarct dementia Parkinson's disease, secondary dementia related to Alcoholism, depression and so on were excluded on the basis of clinical history, neurological examination, laboratory tests and so on. The criteria that were used to include patients with AD were 1) slow progressive decline of intelligent attributes, 2) a score on clinical dementia rating scale of more than 0.5, 3) a Short Portable Mental Status Questionnaire (SPMSQ) of less than 20 out of 30 and 4) a score of 7 out of 18 on the Hachinski scale.
Out of 278 patients that was sort; only 20% satisfied these criteria 56 patients were 55 years. 76 and 66 patients were from 55 to 59 years and 60 to 65 years respectively. All the controls had more than 20 on SPMSQ Score. A number of first degree relatives were not examined because they were dead. The family history information was obtainedby interviewing next of kin of the patient or control. (8) (9) (31)

The results of this study indicate the heterogeneity of the issue. The notion that early onset is genetically engineered is becoming an accepted idea. The age of onset andgenetic linkage support the idea that an autosomal dominant gene may be involved.Among the findings of the above study, is that the onset as well as segregation analysis does not fit the age of onset of AD before 65 years. The comparison with late onset, suggest no clear distinction in the frequency of the autosomal dominant allele between late and early onset AD. The mechanism that determines who is susceptible to AD may be thesame for early as well as late onset. The age of onset may be influenced by other genetic or non genetic factors. In this study the evidence for multi-factorial effect is weak in the study of early onset. Proband are selected based on the fact that they belong to the family that is already known to have the disease in more than one family member. However, there is a risk of bias on both representativeness and severity. Again DAT being an age dependent disease, the gene for late onset is hardly expressed before the patient dies. (14) (17) (31) (32) (33)

Around 1987, four groups of investigators did research on the familial risk of AD for 125 individuals that have been diagnosed at autopsy for having AD. They found among other things that there were differences in early and late onset AD. They also found that early onset with at least one affected

relative increases the risk of first degree family members as they approach the age of 90 years with about 50% chance of AD. There are similarities between family members and the age of onset. Just like many other investigators, this group of investigators found that genetic factors, if operative, are essential in the early onset of AD. Family studies provided age-specific occurrences of AD among relatives that has never been found in other investigations. (50)

We know that when people approach their later years, they experiences memory loss (senility) and so it becomes important to distinguish memory loss caused by AD from senile dementia. Two investigators Sjogren in (1952) and Larsson in (1963) studied presenile AD and senile dementia respectively. The studies helped to differentiate AD and senile dementia on the basis of age at onset as well as clinical signs that were present or absent. They found that AD onset was roughly before the age of 70 years with the presence of clinical signs and called it presenile dementia. Senile dementia was found to beat a much later age beyond 70 years of age with the absence of clinical signs. Considering the two studies, the senile dementia like the AD is believed to be transmitted by an autosomal dominant gene. But unlike the AD, which can occur approximately before orlater than the age of 70, the Senile dementia is much later around the age of 90 years. (52) (64) (65) (92) (93)

However, it should be noted that in Larsson's study, it was found that a case of secondary senile dementia occurred as early as 52 years. This is contradictory to the age later than 70 years that was mentioned above thereby showing a bias to the study's age boundaries. With the criteria used in diagnosing AD today, 25% of these diagnosed of AD would not have been diagnosed as above. This would have affected the results of the studies. Nevertheless, the studies that were done by Sjogren and Larsson; with the criteria used today in AD diagnosis, 25% of those diagnosed as having AD, would not have been diagnosed as such. This would have affected the results of the studies. (47)(52)(92)(93

Another series of studies on AD and senile dementia was done by Heston and Mastri, 1977; Heston and White, 1978; Heston, 1981; Heston and Mastri again in 1982 ; Heston 1984; and so on. These studies found that there appeared to be two illnesses; familialearly and late onset AD which is contradictory to the studies of Sjogren and Larsson above that suggested that there is a clear age cut between AD and senile dementia. The study of 1981 by Heston also revealed that the risk of AD to early onset of first degree family increases with age up to 50% by the age of 90 years. Also in the family members of those examined, the age of onset had a significant resemblance in those family members studied. Also secondary cases seem to manifest at ages considerably older than those identified individuals that had been diagnosed with the disease. All these results observed by Heston did not support the explanations of autosomal dominant gene interpretation. (47) (50) (52) (54)(48)

Heyman and colleagues using NINCDS/ADRDA standards studied the families of 68 AD cases. All the patients had an age of onset of the disease before the age of 70 years. Unlike Heston , Heyman did not find greater familial AD risks in early onset cases, but his maximum age limit of 70 years at onset, may have disrupted the meaningful comparison that would have been obtained otherwise. Heyman found in

his study that proband showedonly mild to moderate disease. (48)(53)

Breitner and Folstein in 1984 studied 78 subjects with 33 age and sex-matched controls; in their studies of early, middle, and late stages of AD classification. They categorized the proband with the middle stage. Out of those 78 subjects, 42 had the middle stage AD symptoms, 8 had not shown the middle stage AD symptoms but hadbeen ill for less than 4 years; and 12 had also been ill for more than 4 years but had not shown the symptoms for middle stage AD. The rest are not yet ill and lack symptoms. Breitner and Folstein found that there is a difference in AD risks among first degree relatives of probands when compared with subjects lacking the symptoms. While the relatives of the middle stage proband showed 50% of AD by age 90 years, the control relatives showed a life time risk of only 8%. (51) (54)

All these studies combined showed that autosomal dominant inheritance, even though it is not certain, may play a key role in genetic transmission of large numbers of AD cases. However, confirmation of this theory of autosomal inheritance is still inadequate. Problems have been shown by late expression of AD and also the difficulties inthe diagnosis of the disease.

CLINICAL STUDIES OF FAMILIAL ALZHEIMER'S DISEASE

The Familial Alzheimer's Disease (FAD) is the type of Alzheimer's Disease (AD) that is consistent with hereditary and or genetic transmission. In FAD there are at least three genetic loci that are important or responsible in AD transmission. These loci are useful in diagnosis of AD as well as testing. The usefulness and limitations of genotyping genetic loci, is necessary for clinicians to use in diagnosis of AD in 50 to 60 year old patients. The three genetic loci are: 1) the APP gene that has a series of mutations, causes individuals to develop AD 2) the APOE gene that has E4 Allele, in which genetic variability is a predisposing factor for late onset AD 3) the center of the AD disease mightbe said to be a fibrillar B amyloid process. All the above are used as of today in AD diagnosis. (34)

In APP using the amino acids (valine, isoleucine, phenylalanin, glycine, isolycine, methionine, asparagine and leucine) gene-based diagnosis have four mutations that has been identified namely 1) APP 717 Val --> Ile, 2) Val --> Phe, 3) Val -->GLy, and 4) APP 670fflys/Met --> Asn/Leu. All these mutations cause autosomal dominant AD. The age of the onset is between 48 and 60 years. The APP 717 Val -->Ile is the most common form of mutation that is seen in families of AD patients. APP 717 valine to isoleucine mutation has been found in several families with early onset familial Alzheimer's disease. It is important to note that this variant has not been found in the general population of caucasians, even though it may be a common cause of AD in other populations. The mutations of the B- amyloid protein section of the APP gene (exon 16 and 17) might be likely causes of other cases of FAD. Investigators tried to find out about the mutation on the B-amyloid sequenced exon 16 and 17 by using PCR direct sequencing. The PCR conditions were 94 degrees centigrade for 10 minute to denature proteins, then 35 cycles of 60 degree centigrade for 1 minute, followed by 72 degrees centigrade for 3 minutes and 94 degrees centigrade for 1 minute 30 seconds. Finally a single cycle of

72 degrees centigrade for 10 minutes was conducted in amplifying the exons. Exon 16 as well as 17 using PCR primers, failed to reveal any mutation on either exon. However, the investigators noticed a band shift on exon 17 with families of an APP 717 mutation. Since this is contrary to the belief that B-amyloid protein contributes to FAD, the investigators offered two possible explanations for these findings which explains the deviation from general belief The first explanation they offered was that there are mutations elsewhere in the APP gene which are Alzheimer's disease causing (Pathoge nic). The second explanation was that the families have mutations in genes other than the APP gene. It remains very possible that other sequence differences in the APP gene might exist between affected and unaffected individuals either in examined sequence of the amino acid or in the promoter site on Deoxyribonucleic Acid (DNA) at which Ribonucleic Acid (RNA) polymerase attaches to the operon.(33)(45)(46)(74)(75)

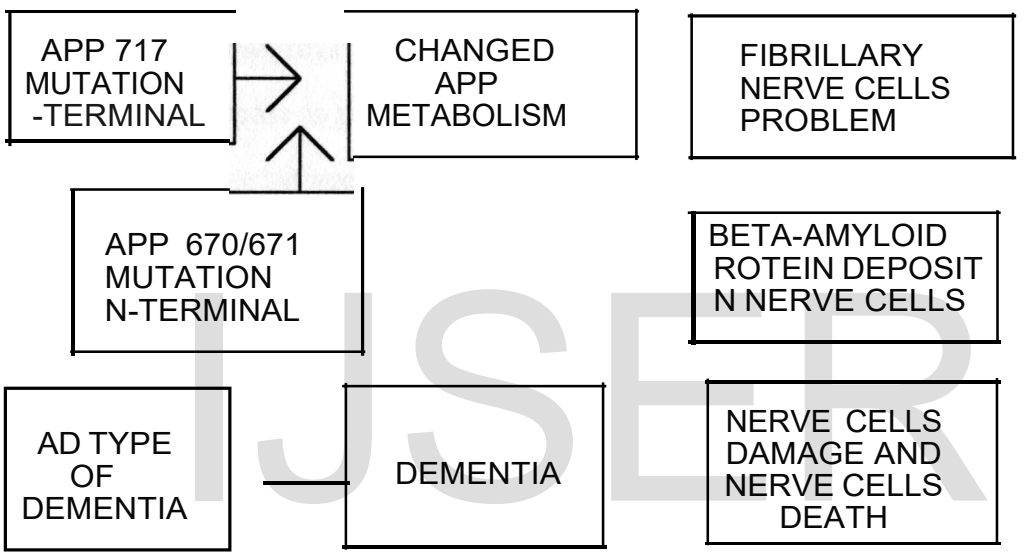
The substitution of the Val with Ile or Gly at codon 717 results in an increase ofB- amyloid precursor protein deposit because of the changes in APP processing, and the variation in the sequence around this position may result in an increase of APP messenger RNAs translation causing AD by a route similar to that which is believed to cause Down'sSyndrome. However , in the absence of any mutation , deposition of small amounts of B- amyloid still occurs to a small extent under normal conditions. The amyloid molecules which are obtained from the brain of diseased AD patients showed N- and C- terminal (NH2 and COOR terminal) heterogeneity suggesting an alternate pathway. The actual mechanism ofB-amyloid production and release is currently still uncertain. The mutation of the APP gene that causes amino acid substitution from Val to Ile, is close to the C- terminals of the B amyloid precursor protein. The screening of other cases of FAD, revealed an unrelated family in which this variant occured suggesting possible mutation onthe APP gene causing AD. (74)(79)

On chromosome 14 based diagnoses, the locus on chromosome 14q24 segregateswith the AD. In this case, the first degree relatives could be offered DNA - based geneticcounseling based on the analysis of linkage of the chromosome.The family members are tested for alleles of particular markers, in order to determine which family members are affected. Quite unlike other related dementia, the pathogenic locus has to be identified on chromosome 14 genes before counseling the individual. (50) There has also been evidence of age to chromosome 21 in some analysis but other analysis failed to obtain evidence for a locus on chromosome 21. This led to the suggestion that there might be some loci that are predisposed to EOAD on chromosome 21. Some investigators said that the APP gene is not the site for the segregation because of failure to observe co-segregation between the APP gene and AD locus. However, other researchers feel that it is possible that non co- segregation may have occurred in families in which there is no causative locus on chromosome 21. This is because in a single family there is both evidence for linkage to chromosome 21 markers and recombination between the APP gene and AD locus. In the APOE - based diagnosis, the APOE E4 allele is the major factor for late onset AD. If a patient has been found to have the E4 allele, the patient is likely to develop AD but if the patient is diagnosed with two E4 alleles, the patient's chances of developing AD is increased to 90% out of a 100% by the age 75 years. The APOE locus has been found on chromosome 19. It has 3 alleles namely

APOE (E2), APOE (E3), and (E4) . Investigators found that 80% of familial and 64% sporadic AD that is associated with late onset had at least one APOE-E4 when compared with the control subjects that had only 31%. As a result of this finding, the investigators believed that the E4 allele is an important factor in the etiology of more than half of all AD. However, the mechanism by which the E4 allele causes AD is yet unknown but the protein encoded by E4 is immunoreactive to the plaques and neurofibrillary tangles that is clearly shown in phenotype. On an invitro test by the investigators, it was discovered that the E4 form of the allele quite unlike E3 and E2 isoforms, had a higher degree of affinity for B-amyloid protein. Investigators using a sample of 42 families found that two E4 alleles are sufficient enough to cause AD by the age of 80 years which suggests that E4 is a major risk factor. (34) (36) (75)

Unlike other apolipoproteins which are produced in the liver, Apolipoprotein Eis produced by Schwann cells of the peripheral nervous system (PNS) and astrocytes and oligodendrocytes of the central nervous system (CNS). Researchers found that in the brains of patients with AD there was widespread degeneration of neurons which lead to axon destruction. The immune histochemical studies of AD brains using antibodies to APOE found that APOE immunoreactivity was present in neurofibrillary tangle and amyloid deposits in both senile plaques and cerebral vessels in AD brains. The researchers obtained the brains at autopsy from 5 AD patients and stored the parietal portion of the brain frozen at -80 degrees centigrade. A section of the brain was cut on a cryostat and fixed in cold acetone for 15 minutes and finally it was subjected to immune histo-chemistry. The rest of the brain was fixed in formalin and processed for standard neuropathological examination. Another portion of the frozen brain of AD patients was processed using a cyostat after which antibody to human APOE was used tostain smooth muscles of the cerebral vessels including astrocytes. The localization of APOE immunoreactivity in astrocytes and other nerve areas in CNS is consistent with previous reports done on rat brain and young adult humans. The immunostaining of all of the AD associated structures strongly suggested that APOE is involved in different types of amyloid deposits and neurofibillary tangles. Like APOE produced by other sources, theAPOE produced by astrocytes and smooth muscle cells could contribute to the amyloid deposits and neurofibrillary tangles. A close look at the diagram on figure I below indicates the pathway of APP. In the introduction, I clearly stated that certain nerve cells are vulnerable and subject to cell death. Here I am trying to diagrammatically explain the process that causes the nerve cells to die. (27) (34) (56) (57)

SPECULATED PROCESS OF AMYLOID PRECURSOR PROTEIN



The diagram is modified from "Alzheimer's disease: Clinical MolecularGenetics" page 243

FIGURE 1

FAMILY WSTORY STUDIES OF THE CUMULATIVE RISK FOR ALZHEIMER'S DISEASE

The cumulative risks for Alzheimer's disease; increases with family members of first degree relatives. However, whether or not genetics plays a part is yet unclear. As a result of the above, some investigators still believe that AD is multi-factorial and not just genetic. In Mendels' view on segregation, marriage between two affected heterozygous dominant trait individuals, suppose to produce offspring of three affected and one unaffected person. However, the marriage between affected and unaffected people has a fifty-fifty tendency.

Many investigators of AD believe that it is not genetically engineered. The fact that the Mendelian

pattern does not exist in families of AD patients might indicate that AD is not inherited. The problem with this pattern is that, if the disease has a late onset, the patient might die of other causes before being diagnosed of the illness. The study of the pattern in late onset is possible only if some of the population being investigated lived through the risk period. There are three ways this type of study can be conducted, namely: I) the family history method, 2) the family study method and 3) the study of health records. (85) (91)

The family history studies method, include interviews with knowledgeable family informants to the disease. The life-table method takes into account the decreasing numbers of individuals at risk for the disease as their age increases. The family history study method has other attributes that potentially can provide information suggesting the presence of a particular mode of transmission. The family history method demonstrates familial factor and not genetic factors. Unlike the family history method which relies on historical accounts from living informants and does not necessarily include direct examination of relatives, the family study method includes clinical examination and or field investigations of each living relative at risk. The last method which is the study of health records relies on obtaining many records that includes records of death certificates. This method is usually difficult to obtain as well as time consuming. The family study method and the study of health records are both very reliable even though they are not practical. As a result the family history method is often the only practical option used. Butthe problem with this data is its reliability (58) (85)

Since the family history method was the only practical method, its reliability is very important. An investigator studied the reliability of such data by using two approaches. (1) The first thing they did was to standardize the family history techniques and then check thereliability across multiple informants. The importance of standardizing this technique is necessary but not good enough to be used by itself without being combined with other methods. (2) The second thing they did was to compare their results with several previous investigators and if the results are consistent, that can help to establish its reliability. (59) (85)

The investigators obtained the family history of 27 proband AD patients and 22 non-demented control subjects with ages that range from 50 to 85 years and 46 to 79 years respectively. Using the current diagnostic criteria, the 27 AD patients were obtained. An Alzheimer's dementia and risk to family members' questionnaire was used along with a newly devised dementia questionnaire. The AD probands initial informants were family members who were directly involved in AD patient care. The control subjects served as first informants for their own family. The second informants that were knowledgeable about the history were located and independently interviewed for 20 AD probands out of the 27 proband (74%) and 14 controls out of the 22 controls (64%). (58)

The reason for this study was to find out if there was agreement between multiple informants. Among their findings were that reliable information about dementing illness may be obtained with the family

history method. However, in order for this to be possible, a standardized technique has to be used. In most cases the family history method has been supplemented with the family study method. This is because the family history method tends to give false positives and also underestimates the amount of the actual illness. (58) (59) (73)

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Since the family history method was the only practical method, its reliability is very important. An investigator studied the reliability of such data by using two approaches. (1) The first thing they

did was to standardize the family history techniques and then check the reliability across multiple informants. The importance of standardizing this technique is necessary but not good enough to be used by it without being combined with other methods. (2) The second thing they did was to compare their results with several previous investigators and if the results are consistent that can help to establish its reliability. (59) (85)

The investigators obtained the family history of 27 proband AD patients and 22 non-demented control subjects with ages that range from 50 to 85 years and 46 to 79 years respectively. Using the current diagnostic criteria, the 27 AD patients were obtained. An Alzheimer's dementia and risk to family members' questionnaire was used along with a newly devised dementia questionnaire. The AD probands initial informants were family members who were directly involved in AD patient care. The control subjects served as first informants for their own family. The second informants that were knowledgeable about the history were located and independently interviewed for 20 AD probands out of the 27 proband (74%) and 14 controls out of the 22 controls (64%). (58)

The reason for this study was to find out if there was agreement between multiple informants. Among their findings were that reliable information about dementing illness may be obtained with the family history method. However, in order for this to be possible,a standardized technique has to be used. In most cases the family history method has been supplemented with the family study method. This is because the family history method tends to give false positives and also underestimates the amount of the actual illness. (58) (59) (73)

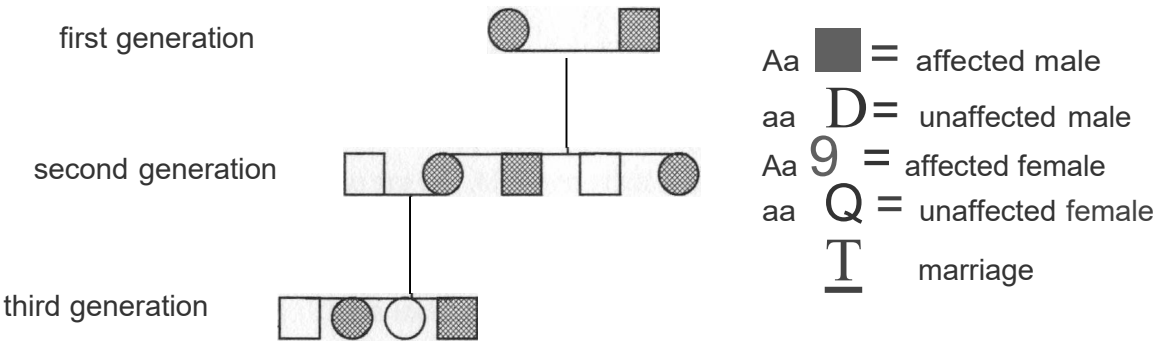
Cumulative risk studies of first degree relatives of AD probands have produced similar results for relatives at risk, their associated cumulative risk and standard error. Several studies, of all first degree relatives or all their parents and siblings have been madewith the cumulative risk ranging from 39% to 49%. In some of the most recent studies, the cumulative risk of the siblings was found to be somewhat higher than that found in their parents. This higher rate might be due to better quality of information regarding siblings when compared with their parents who have been dead before the data was collected. I suggested previously that early onset AD may be used to identify a more familial type of AD. Few studies can actually or successfully distinguish cumulative risk estimated between early and late onset relatives of proband through hypothesis testing. The investigator looking for cumulative risk studies has found that risk of early onset probands is higher than that of late onset probands. Two studies were done on sex differences in cumulative risk, however, in both, females showed greater chances of cumulative risk in comparison with males even though statistically it was non significant.(16)(27)

Investigators trying to determine the cumulative risks studied 22 subjects that were rigorously diagnosed of having DAT. Also in their studies, they had 24 non demented control subjects. However, if an

autosomal dominant gene is involved in the transmission of DAT, 50% of first degree relatives of those with DAT are expected to have an affected genome to develop DAT by the age of 90. Nevertheless, the result of the investigation wasthat 67% showed the cumulative risk for first degree relatives of DAT by the age of 86 years and 23% of control also showed the commutative risk by age of 86 years. In both the 22 subjects and 24 controls, secondary cases like forgetfulness that is consistent to their day to day activities but does not render the subjects incapable of other daily activities was used in obtaining the 67% and 23% respectively However , since DAT can be imitated by other conditions, clinical diagnosis of DAT is very difficult. When the investigators removed the secondary cases and installed the standard criteria, the control subjects at risk still remained at 23%, where as the 22 demented subjects decreased to 41% both at age 83. This finding tends to support the hypothesis of autosomal dominant inheritance when you consider the 67% obtained by investigators. The 41% obtained is very close to the 50% that supports the autosomal dominance inheritance. (33) (54) (55)

Other investigators found that the AD like illness affected about 50% among first degree relatives. As was mentioned above, the risk among AD patients when compared with the control was much larger and approaches 50% with late onset AD especially after the age of 70 through mid 80s. In one investigation, it was found that no new cases appeared beyond the age of 87. The result of this investigation conflicts with the studies done by Heston in 1981. However, the reason for the conflict, why familial risk is a function of proband age of onset is not yet clear. In any case, it may be the result of differences in obtaining and discovery methods of probands according to one investigator. (40) (41) The data that is obtained from direct family study and health records are in most cases more accurate than those obtained through the family history method. Also, since AD is typically a late onset disease, most of the secondary relatives (in other words all parents) will be dead at the time of study. Inspite of the fact that the family study and health record are the most accurate; the family history method is often the most practical technique for FAD. It is important to note that the validity of family history diagnosis ofAD in relatives is still unproved. Also the family history study results must be limited dueto the small samples that are available in the study condition. (85)

A marriage between two affected pedigrees



The diagram is modified from "Atlas of the face in genetic disorders"
Second edition page 29 1997 Reference #91

Mendel segregation pattern does not hold in AD

FIGURE 2

GENETIC LINKAGE STUDIES

The view of Mendel segregation does not hold up genetically in an AD pedigree (family tree) . The genetic linkage of AD to DNA markers on chromosome 1, 14, 19 and 21 was studied in several families where the disease has been inherited as an autosomal dominant trait. Knowing that AD is a neurodegenerative disorder with a complex genetic etiology, it is not likely as suggested by complex segregation analysis that one dominant allele can explain a large proportion of cases with AD. Linkage studies have suggested genetic loci for FAD on four different chromosomes that I have mentioned namely chromosome 1, 14,19, and 21. (15) There are four genetic loci for these chromosomes that may be responsible for AD. These are the APP gene that is found on chromosome 21, the APOE that is located on chromosome 19, and 2 unidentified loci that are located on chromosome 1 and 14 respectively. These loci, seem to be associated with the Alzheimer's disease onset which are early (before the age of 65) and late (after the age of 65) onsets. By onset, I mean the first cognitive symptoms. (4) (7) (10) (68)

Chromosome 1

In 1995 researchers discovered a gene on chromosome 1 that might be responsible for AD. The chromosome 1 gene is very similar to that found on chromosome 14; gene S182. The protein that this gene encodes for might have similar functions and if so, might help to explain the biology of AD. This gene on chromosome 1 was discovered in a Russian family in 1995. Investigators are suggesting that defects in the gene found on chromosome 1 (STM2) as well as that found on chromosome 14 (Sl82) might be responsible for making the increased amount of B amyloid which may be the triggering factor for nerve cell damage and death causing dementia of the Alzheimer type. A lot has been said by investigators about the clustering of AD in families which is indicative of inheritance; however the heterogeneous and complex nature of AD makes it highly inconclusive to say that AD is genetics. The clustering could also be by chance. (9) (30) (45)

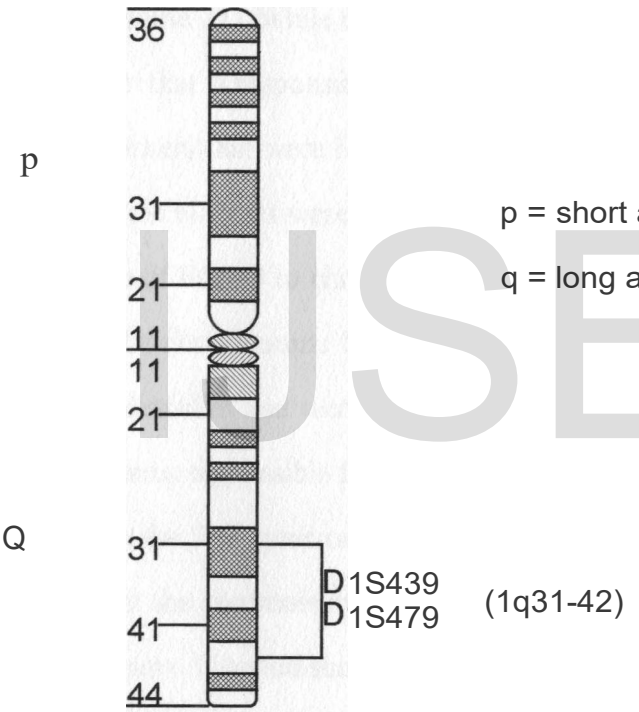
Investigators are maintaining cautions about this kind of discovery because the APP gene that is located on chromosome 21 when discovered thought that they had found the cause of AD. However, they found out later that their discovery accounted for only 2% to 3% AD cases. They also found that many more cases of AD were caused by a chromosome 14 defective gene. (4)(29)(30)

The study of chromosome 1 that was done on Russian families, showed a mutation on the chromosome that was neither on chromosome 14 or chromosome 21. However, with the help of genetic markers the investigators were able to accurately pinpoint the gene with the help of (S182)

and also localized the gene to chromosome 1.

The investigators sequencing the gene on family members of the research group found that the family members with AD all have the same mutation on the gene while unaffected family members did not have the mutation. The family ancestry was traced to two neighboring villages in Russia. I am sure that researchers will introduce soon this mutated gene into mice with the hope of creating new animal models which could be helpful in understanding AD as well as therapy that might be necessary for AD. (30)

DIAGRAM OF CHROMOSOME 1



The diagram is modified from "The science of Genetics: An introduction to heredity" fourth edition page 70 1980.
Reference #46

FIGURE 3

a) Chromosome 14

Trying to understand early onset AD, investigators had studied extensively the effect of the chromosome 14 mutation on AD. Also linked with early onset AD (EOAD) is chromosome 21. While using linkage analysis to search for the genome, chromosomal region that is responsible for FAD genes, a highly significant positive clue was obtained on markers that were found on chromosome 14 in early onset. In population based studies, multiple markers were tested for chromosome 14. However, there is strongevidence for linkage of EOAD to chromosome 14 in many studies of chromosome 14, but in other studies of the chromosome 14 linkage of AD, it was not associated with EOAD. The result of several studies that were considered on both the genetics of FAD and the pathogenic mechanisms responsible for the disease indicated that EOAD can be caused byeither a mutation on the APP gene or a mutation on the chromosome 14 locus. There is also a possibility that the chromosome 14 locus shows a distinction of APP process from pathogenic mechanisms. The lod scores (which is a statistical method of analysis) of most studies suggest that the early onset FAD pedigrees, when considering pair-wise linkage analysis have a mutation that is linked to loci D14S43 and D14S53. However, marker allele frequency is unpredictable when calculating lod scores with single or unpaired pedigrees. (4) (12) (15) (35)

The importance of the chromosome 14 discovery is that four independent groups of researchers observed this chromosome in several families. Each group found that there is agenetic marker located on the long arm of chromosome 14 that is linked to early onset familial Alzheimer's disease. According to these four groups of investigators , chromosome 14 unidentified gene may account for 70% of EOAD and the APP defect on chromosome 21 may account only for 5% of the cases. With this kind of finding, it is important that solving the problem of identifying the gene on chromosome 14 may be important in solving the problem of EOAD. Before chromosome 14 was found, geneticists had realizedthat there is more to AD than APP involvement. In one of the families' studies of AD, the D14S43 on the long arm of chromosome 14 markers were significantly linked with Alzheimer's disease. In 1992, St. George-Hyslop and his associates found a significant linkage in 6 families out of the 21 families they studied. He also added that there is aleeway for error in the way the experiment was conducted. (40)

Hardy Mullan and Fiona Crawford reported in 1992 that chromosome 14 markers were significantly linked to FAD in one family that had EOAD. Their report was supported by several other independent investigators. Another group of investigators studying Belgian families that include 6 or 7 generations of patients who developed AD at the age of 35 found that a chromosome 14 linkage was noticed on 2 of the families. An investigator in this group, by name (Van Broeckhoven) said that there was a single family member who did not jointly inherit both the D14S43 marker which is the disease locus forhis or her parents. Schellenberg placed the marker on chromosome 14 between D14S52 and D14S53 which seems to be poorly mapped when compared to other investigators mapping ofD14S43 and

D14S53. Schellenberg and associates 1992 also found that the chromosome 14 defect does not occur in a special group of U.S. and Russian families whose German ancestors settled along the Volga River in Russia in the mid-18 century. (3) (35) (41) (42) (68)

Knowing that amyloid protein is involved in AD, investigators tried to link the chromosome 14 genes to the amyloid picture. They tried to link the gene that encodes for the proteinase inhibitors alpha-1-chymotrypsin and antichymotrypsin that are on chromosome 14. These proteinase inhibitors are just two of many amyloid precursor proteins that are involved in the neurofibillary plaques. Another gene that encodes for cathepsin, is known to cleave APP which could lead to the accumulation of amyloid plaques. However, the gene that encodes for proteinase inhibitor and cathepsin, falls outside the chromosome region that is linked tightly to AD. (40)

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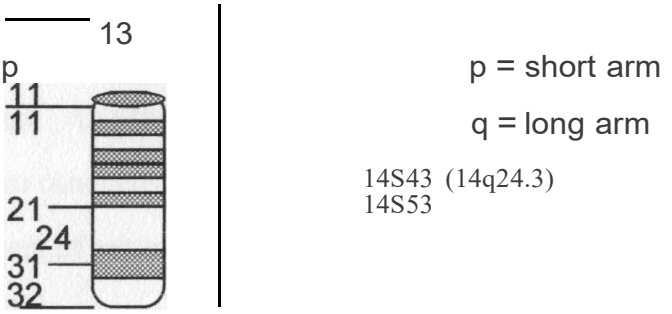
DIAGRAM OF CHROMOSOME 14



The diagram is modified from "Encircling a mechanism in Alzheimer's disease" J of NIH research page 48 1992.
Reference #40-

FIGURE 4

c) Chromosome 19

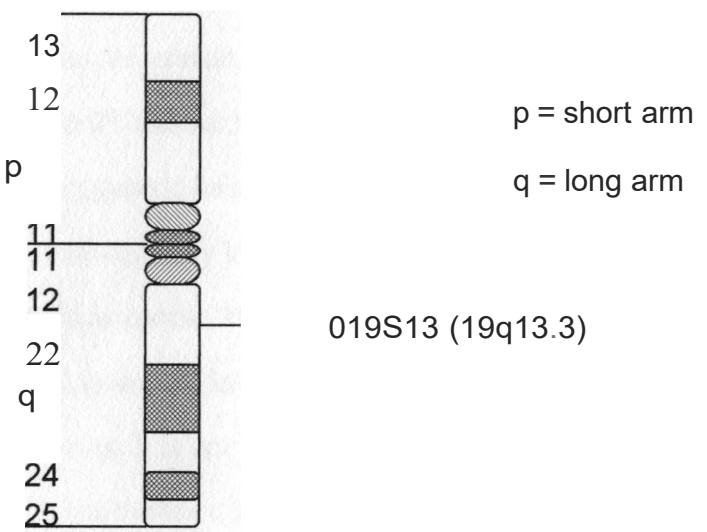
The fact that some scientists or investigators realized that APOE gene mutations were responsible or might be responsible for FAD caused some researchers to search for other gene loci that might be responsible for AD. The APOE locus contains four exons that code for 299 amino acid plasma proteins. It was then that linkage to chromosome 19 was suggested in late onset pedigrees. This linkage is associated with theE4 allele of apolipoprotein E. This APOE gene was found to be bound to B-amyloid and therefore might be set apart from abnormal protein. Apolipoprotein E gene is located in areas of the observed linkage. An experiment that pointed toward APOE; high-affi nity- protein binding studies were conducted through the use of electrophoresis by investigators. The component of cerebrospinal fluid (CFS) was seperated by means of electrophoresis in both Alzheimer's patients and normal subjects. The researchers did thisby blotting their sample of the fluid onto gel matrix loaded with synthetic B-amyloid, fragments of B-amyloid, or control peptides. Later, upon examination of the substance stuck to the amyloid, they found that most of the proteins were identical in AD patients and the control subject except for a band that was either absent or in a high concentration in AD patients. The control subject had moderate amount of the protein which was later discovered to be APOE. The same investigators further stained tissues from the brains of people who died of AD. Using diluted antibodies to APOE, the researchers found staining on plagues and tangles. This staining did not occur in the brains of non-Alzheimer's patients, demonstrating that immunochemical staining only occurs if plagues and tangles were present. These researchers also found that the gene that encodes for APOE, was located on the long arm of chromosome 19 which has been found to be linked with late onset FAD.

This research also found that an E4 isoform type of APOE was significantly linked with late onset FAD and sporadic AD which constitute the bulk of the AD cases. Some researchers analyzing serum from 12

patients diagnosed with probable AD, 48% of these individuals have the E4 allele when compared with 13% of their spouses that have the E4 allele. So the researchers concluded, that when one has the E4 isoform of APOE, in a family, that is very likely that those individuals can come down with FAD. However, if you have the gene and no known family history of AD, the person is more likely to have a sporadic AD. (40) (43) (44)

According to Corder, 1993, late onset FAD which is associated with Chromosome 19, showed that all E4 homozygote's, develop the disease by the age of 80 years in families that had APOE mutations. (Hardy 1993). Investigators also believed that there is association between E4 and sporadic AD. The APOE locus is a gene that contains four exons that code for 299 amino acid plasma proteins. Some investigators, examining family histories of individuals positive for AD and those negative for AD, found distorted APOE alleles in the families of positive individuals. All allelic distribution of family history of positive siblings at risk were found to have an excess of the E4 allele. The E4 allele also differs from family histories of control siblings. The ages of the affected and unaffected siblings were similar. This result shows that the false account of allelic frequency of unaffected siblings was not due to their mean age being below the age of onset. Lod scores however, did not suggest linkage to the APOE locus. Nevertheless, analysis of variance suggests that the APOE locus enhances the rate of progress of the disease but did not accurately predict the disease. The investigators also noted that variation on this locus is not enough to cause the disease. (Note: The control was obtained by digesting APOE PCR products with restrictionenzyme "Cfol") (7) (29) (39)

DIAGRAM OF CHROMOSOME 19



The diagram is modified from "The science of genetics: An introduction to heredity "
Fourth Edition page 74 1980.
Reference #46

FIGURE 5

d) Chromosome 21

Alzheimer's disease similar neuropathological features as seen in older patients with down syndrome, which is associated with chromosome (trisomy) 21, had led some investigators to believe that a gene on chromosome 21 might be responsible for Alzheimer's just like Down's syndrome counter path. However, their beliefs were shaken since it is not certain that those Down's syndrome patients develop dementia despite the high incidence of Alzheimer's like neuropathology. Also heterogeneous groups of other conditions that included normal aging mimic some neurochemical and neuropathological aspects of Alzheimer's. This, as a result, makes it impossible to conclude that there is an association between tisomy 21 and Alzheimer's. (18)(19)(66)(78)

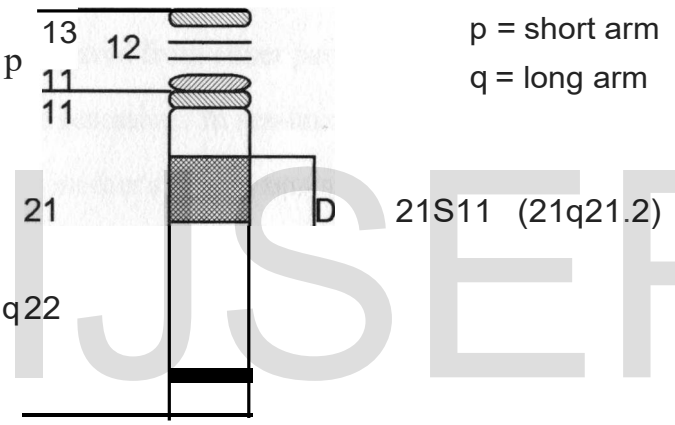
Investigators, using genetic linkage analysis with DNA makers studied four kindred which had been histologically known to have FAD. They found that even though FAD gene is located on chromosome 21, it is not located in the 21 or 22 regions as in the case of Down syndrome. Instead, the investigators found the gene of FAD to be located in21Ql 12 to 21Q222. However, it is not known whether these individuals experienced AD- like neuropathology. The more recent studies of chromosome 21 linkage to AD revealed a combined locus ofD21S1 and D21S11 among other loci that have been found earlier onthe proximal long arm of the chromosome. These loci complement and extend the studiesof chromosome 21. In the first study done by Tanzi and associates in 1988, it was found that the chromosome 21 proximal long arm that was associated with AD were D2l S1, D21S11, D21S4 and D21S13. However, no gene has yet been assigned to the proximal long arm of chromosome 21. (11) (19) (78)

Like chromosome 14, chromosome 21 is also associated with early onset AD. An investigator found that chromosome 21 has a mutation that is associated with EOAD, even though many early onsets show no ties with AD. The APP gene was found to be on chromosome 21 and B amyloid protein is present at the core of the plagues foundin the brains of AD patients. Investigators found mutations in members of two unrelated families with early onset FAD. Another mutation at a separate location on the APP gene was found in another family with the same form of the disease. Investigat ors, on a certain study of EOAD and LOAD found that EOAD, displayed positive evidence of linkage to loci D21Sl/Sl 1, and D21S 13/S16. It was found to affect pedigree of EOAD but was excluded in pedigrees of LOAD.

A genetic linkage was revealed on the proximal long armof chromosome 21 in four different families with EOAD. The demonstration of a genetic heterogeneity mask is apparent in data from different family studies that could not be put into common use for linkage studies. A study of a single family with FAD linkage to chromosome 21 showed no recombinant event between APP gene and the disease locus, but a direct sequencing of APP gene revealed a point mutation at 717 codon outside the C-

terrminal of the B-amyloid sequence. Also identified in the N terminal ofB amyloid, by a study of pedigree, is APP 670 and APP 671. The reason why these three APP genes are involved in FAD is still unclear. Also the function of APP and B amyloid in normal metabolism is not fully understood. However, APP mutation and B amyloid sequence areclosely related.(5) (29) (85) (88)

DIAGRAM OF CHROMOSOME 21



The diagram is modified from "Physical Mapping around the Alzheimer's disease locus on the proximal long arm of chromosome 21" Am J Hum Genet page 317 1990.
Reference #78

FIGURE 6

MOLECULAR GENETIC STUDIES

In considering molecular genetic studies, it is important to examine the three modes of common genetic inheritance namely autosomal recessive inherit ance, sex-linked inheritance, and autosomal dominant inheritance. In autosomal recessive inheritance, both parents of the individual have a recessive gene that is transmitted to the offspring but if only one recessive gene is received from either parent, the gene is hidden from manifestation thus said to be recessive. In sex-linked inheritance, the male inherits abnormal genes through the mother's X chromosome that is defective. However, there is no evidence that linked the sex chromosome to AD. Finally, in

autosomal dominant inheritance the individual inherits the disease from both parents and the genes are clearly capable of producing the same phenotypic effects whether the genes are paired or not, quite unlike autosomal recessive inheritance, where the individual does not come down with the actual disease that is transmitted through a defective gene. In order to show a molecular genetic basis for a disease, a sample population consisting of large families withmultiple effected members must be looked at for inheritance patterns and the mode of inheritance. Also selected families should be studied using recent molecular genetics mapping techniques. When identical patterns of a disease, which in this case in AD, show an inheritance, pattern on a deoxyribonucleic acid (DNA) marker, the marker is considered to be linked to the gene responsible for the disease. If the marker is on a different chromosome, then the pattern is random and independent of one another (unlinked).

Knowing that there are two copies of each chromosome contributed by each parent randomly, the marker can be traced from family members to family members through the generations. (14) (63) (91)

In molecular genetics studies, DNA markers are created by cleaving DNA using restriction endonucleases which results in DNA fragments known as restriction fragment length polymorphisms (RFLPs). These (RFLP) are used as markers in linkage analysis and micro-dissection of human genomes. The molecular genetic studies make it possible to detect defective genes to a specific chromosomal region and loci for Alzheimer's disease as well as other medical conditions . With the help of molecular genetics techniques, a moredetailed study of genetics of AD was conducted by attempting to localize the disease gene loci. It was then that the amyloid precursor protein (APP) gene was found to be encoded by a gene on the long arm of chromosome 21 which was later associated with EOAD.(38)(66) (67)(91)

Humans have 46 chromosomes which have more than 200,000 genes. This makes it difficult to link a DNA marker to a particular gene. Investigators found APP gene that is on chromosome 21 when isolated and sequenced, was found to code for B amyloid precursor protein gene (B APP) that has several mutations which are linked to AD. The most common mutations are found on codon 717. This results in a change of valine to isoleucine, valine to phenylalanine and valine to glycine. Another locus was also found onchromosome 14. Even though investigators have mapped the locus, the specific gene has not yet been identified. Both chromosome 14 and 21 are believed to be associated with AD. A third locus was also found on chromosome 19 near the centromere that is associated with late onset patients. The APOE gene has three forms: E2 , E3, and E4. The E4 form is believed to be associated with LOAD. APOE locus is encoding a 299 amino acid plasma protein. (63)

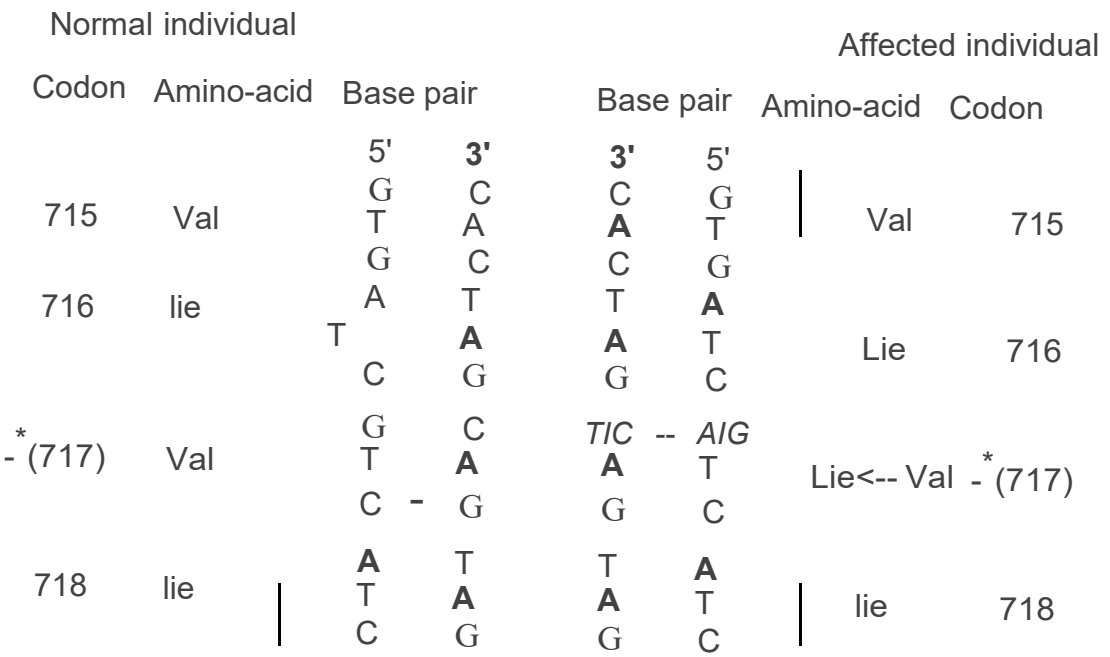
The abilities of using a modem recombinant DNA process of polymerase chain reaction (PCR) and others have increased interest in genetic studies of AD. As I stated earlier, the gene that is involved in coding for amyloid precursor protein core of senile plaques was localized to human chromosome 21.(51) In some studies, the subjects at risk were screened for AD genetically with polymerase chain reaction-induced mutation restriction analysis (PCR- IMRA). The DNA of patients known to be heterozygous for Phe-717 mutation were sequenced. The mutant alleles were reproduced with PCR technique in all tested positive controls. Among those tested, several were found to have Phe-717 mutation. Many of unrelated non-AD controls were also tested by PCR- IMRA.

In all the controls, none of the alleles were positive for the Phe-717 mutation. An investigator screening FAD kindred members for the APP Ile-717 mutation using more conventional method (RFLP analysis), and the same investigator also screening APP Gly- 717 mutation using an additional PCR-IMRA testing found that one patient was heterozygous for the Ile-717 mutation, while the rest showed neither Phe-717 nor Gly- 717 mutation . Another investigator tested APP Ile-717 mutations by RFLP analysis, found that two samples were heterogeneous for APP Ile-717 allele. Using direct sequencing of the APP gene, it was determined that none of the samples were positive for APP Gly allele. (38) (45)

Sequencing studies on the APP gene revealed an observation of mis-sense mutation in the APP gene of at least two FAD pedigrees. This raised the question of technical errors and the possibility of errors in the diagnosis and non paternity type situations. Earlier the researchers observed the recombinant events between APP and the FAD gene in pedigrees showing evidence of co- segregation with other chromosome 21 markers. The APP gene was then searched for any signs of mutation on smaller pedigrees by the investigators. They did this by encoding the entire sequence exclusively from exon one through eighteen. The 3' (three prime) untranslated sequences were investigated by direct sequencing of PCR. DNA sequencing of exon 17 using PCR revealed a transition on affected individuals from C to T. This caused an amino-acid substitution from val to Ile at codon 717. A sequence difference of C-T was found in affected members of the FAD pedigrees. The investigation of the remaining pedigrees were confined to exons 16 and 17mostly exon 17. (90) (63)

35

A Diagram of the APP 715 through 718 codons showing an example of 717 mutations



In the above diagram, the transition of C to T that caused amino acid substitution of valine to isoleucine

The diagram is modified from page 705 of the segregation of missense mutation in the amyloid precursor protein gene with familial Alzheimer's

Disease Nature Vol. 349 1991.

FIGURE 7

TWIN STUDIES

Twin studies are very important in the sense that if AD is inherited, identical twins would generally be expected to both have the disease. By identical twins, I mean twins that are genetically identical to one another. However, most identical twins that are alike in appearance are usually genetically identical. Questions that arise concerning the cause of AD may be partially answered with twin studies and its genetic effects. There are two types of twins known to mankind, identical (monozygotic) and fraternal (dizygotic) twins.These twins are born and raised about the same time. If genetics actually does play a role in AD, a monozygotic twin pair should manifest higher concordance for AD than a dizygotic pair, which is suppose to show discordance. (14) (38) (72)

Even though twin studies are a very important study as far as genetics of AD is concerned, twin studies are difficult in that the twins are hard to locate. When located, both members being available for the studies is also a problem. The easiest way of finding the twin sample is by finding volunteers. But the volunteers of that sort are not representative of the population. Also the majority of the twin volunteers are monozygote where as the majority of the twin populations are dizygotic pairs. Also male twin pairs are underrepresented. Furthermore, there is no specific diagnostic laboratory test available at this time. There is no uniformity and agreement on diagnostic criteria. This hinders the twin studies method greatly. The study as a result may be biased meaning that the results of studies are distorted due to a non-representative sample that altered the data. The drawback here can also be low specificity. (60) (61)

Twins can also be obtained by registration. This type of obtainment is available in most European

countries. This method is more representative but its reliance is not known and may allow bias in the results. The registered twins' population, when screened with a follow-up examination and diagnostic evaluation, gives the most representative and more complete study. However, it is very expensive and its use has been limited. Also it has very low sensitivity. (62)

The few twin studies of AD type of dementia was not conclusive. This is because in some studies one twin might have AD and the co-twin may be somewhat cognitively impaired and a diagnosis of concordance will be incomplete. In this case the follow up study is necessary but not always obtained due to old age, death and other factors. This is necessary before concordance estimate would be calculated. The current clinical diagnosis for AD is at 90% over a certain period of time. The final diagnosis of 100% comes at autopsy as I have stated.(60)

Some investigators have suggested that twin studies concordance and discordance isdependent on the age of the pair. Various investigators noticed that twins in their sixties and seventies showed concordance of 20% to 40% whereas the twins in their seventies and eighties showed higher rate of concordance ranging from 60% to 80%. As a result, twin studies concordance and discordance is dependent on the ages of twin pairs being studied. The monozygotic twin pair concordance still remains at 50% concordant rate. (60)

Nee studying 22 of both monozygotic and dizygotic twin pairs in 1986 and 1987 found that at least one member from the 22 twin pairs was diagnosed with AD using NINCDS/ADRDA criteria for probable AD. He also found that monozygotic and dizygotic twin pairs show concordance of 41% for monozygotic and 40% for dizygoticsimilarities between each pair of twins. In the above study, 17 out of22 twins were monozygotic and the remaining 5 were dizygotic. The follow up study 3 years later, showed that the concordance rate for the monozygotic twin group has increased from 41% to 50%. (14) (85) (83)

Kallmann's study of 54 demented twin pairs in 1956 reported a concordance of 43% in monozygotic twins and 8% in dizygotic twins. In 1980, Jarvik did a follow-up study on Kallmann's sample of 10 monozygotic twins and 2 dizygotic twins and found that the concordance rate was 40% among monozygotic and 50% for dizygotic but the number of dizygotic samples was very small and that might have caused the percentage increase indizygotic twin pairs to 50%. (14)(29)(95)(96)

In a more recent study (1991) by National Institutes of Health (NIH) of 22 pairs of 17 monozygotic

and 5 dizygotic twin pairs, has reported monzygotic concordance to be 50%. The Survey done by Bergem and Kringlen of Norwegian Twin Registry records contains mostly women (77%) in their late 70's and early 80's. The study was for both AD and Mult-infarct dementia. This study showed (83%) of concordance rate in monozygote AD. The proband concordance rate in some of those 83% was twice that of dizygotic twins. This result strongly supports the genetic linkage hypothesis of AD. However, caution isjustified because the discovery through the above study was not complete since some of monozygotic co-twins that could be diagnosed with AD were not detected by the records linkage method employed. (60) (62) (84)

The Swedish Adoption/Twin Study (SATSA) did a study of 22 pairs, 7 monozygotic and 15 dizygotic twins. They observed that the monozygotic concordance rates for AD are above 50% or more than double the dizygotic rate. Their ages range from 59 to over 90 years with a mean age of 75 with more women than men. However, diagnostic work- ups are necessary to eliminate false positives from being considered. The United States Study utilizes the National Academy of sciences registry of aging twin veterans. These veterans were between 65 and 75 years of age and inspite of age and sex limitations , the monozygotic concordance rates were about 35% and none or 0% concordance in dizygotic concordant. In 1981, an investigator studying identical adult twins who died at age 74 and 85 respectively, were confirmed as having AD. Both sisters were confirmed ashaving AD at autopsy. Both twins have different onsets. The one that died at age 74 had earlier onset than the co-twin that died of ovarian cancer at age 85. Because of diagnostic difficulties, biases, late onsets of AD and many more variables twin studies are difficult to perform. (60) (70)

CASECONTROLSTUDY

Case control studies have been an important research method for detection of familial and environmental risk factors in AD. In a case control study, the informants have to be close family members or spouse that is familiar with the patients' history of various risk factors. The informants and the patients are also interviewed together in order to obtain the maximum information gathering. The control subjects are free of dementia which will provide accurate information on exposure. Also to avoid under reporting of exposure, surrogates for control subjects were not used or interviewed. (25)

In 1991, four case control studies were done. These case control studies, calculated an Odd ratio (OR) which is the unusual deviation from the normal of family history of dementia and AD as a risk factor. It was found that three out of these four case control studies showed that a family history of dementia and

AD were significant risk factors. However, one study failed to detect this association in 64 patients whereas the other three studies have indicated that a family history of dementia AD is a significant risk factor in a series of those 64 patients with AD. In the study of Chandra and associates, the OR proceeding history of head injury with loss of consciousness was greater than the OR for family history of dementia. However, neither factor was highly or significantly increased in comparison with the control. An explanation for this lack of increase might be due to the differences in the age at onset of AD in these studies. (85)

The study by Chandra, of the 64 patients with AD showed late onset disease (70 years and over). Heyman in contrast to Chandra, showed age of onset earlier than 70 yearsin all AD cases studied. In Amaducci's study, 0.5% of the cases were earlier than 70 years. In the study of Shalet, the age of the onset of proband was not specified. Many findings showed that early and late onset of AD may be a heterogeneous disorder. Even-though case control studies can show proof of familial factors in the disease, the method cannot easily separate the effects of environmental factors from those of genetic factors within families of AD patients. Although enough evidence exists to prove that AD is associated with a family history risk factor, the evidence of these studies cannot alone prove genetic etiology of AD. (16) (26) (27) (94)

Another study was conducted in New York on dementia of an elderly population from various registries for AD which were obtained using a diagnostic method that was based on a relationship between cultural and educational demographics of the New York community. Those chosen also were elderly people that met the criteria for probable AD and those elderly that did not show any probable AD symptoms. However, in both the control and other studies of the patients, the average age of people at risk was higher for the relatives of patients than for those of control subjects. 10% of the relatives of the patient were reported to have AD that ranged from definite to probable. In the relatives ofcontrols, 7% were reported to have AD symptoms using the criteria used for the patients.In the relatives of the patients, the onset is before the age of 70 years. (24) (25) (28) (85)

In the recent case control studies conducted in Australia in 1990, 170 cases of ADages 52 to 96 years were matched with 170 cases of controls. The risk factor interviews that includes perilous health lifestyle, family history, occupational and domestic exposures were carried out with informants (close relatives) . The AD patients were obtained from Repatriation General Hospital Concord and Lidcombe Hospital through the primary care physicians. Control subjects of the same age and sex were obtained through phone solicitation. The controls were given a standard clinical examination involving

neurology of aging tests (which include: auditory-verbal comprehension, test of orientation, vigilance, reaction time, object naming, reading, writing, drawing, and others) . Mini- mental state examinations were also performed. The patients in the control group all lacked evidence of cognitive decline. Of all the variables which include Down Syndrome, Leukemia, thyroid disorder, Cardiovascular disorder and so on, it was found that history in first degree relatives of dementia and probable AD has a significant odd ratio or is likelyto be associated with genetic factors.(72)

DISCUSSION

Alzheimer's disease is thought to be caused by a series of factors but the discovery of mutation in the APP gene in those families that have the disease is a major breakthrough in diagnosing the development of AD. The reviewed literature reveals the influence of genes. Among my findings in the literature review, is the APP gene locus thatis affecting familial and non familial AD cases compared to controls or samples. The mutated alleles are in concordance with the familial AD cases. (21) (39)

The genetic study of AD shows that there are at least three loci that contribute to AD: (A) The deposition offibrillar amyloid (B) The APP gene, and (C) The E4 allele thatis found on the APOE gene. The genetic findings on these three loci are now being used for AD diagnosis. Even though it is not yet evident, my review has focused toward geneticrevolution of AD. However, all the pieces of the puzzle have not fully come together. In the family and genetic studies reviewed, I found that there is a clustering of AD in families with the APP gene and the linkage to chromosome 14 and 19. All the studies performed are not conclusive due to certain limitations. A number of first degree relatives were not examined because they were dead and so the family history information was obtained by interviewing next of kin of the patient or control. Also in those studies, proband is selected based on the fact that they belong to the family that is already known to have AD in more than one family member. This presents a risk of bias on both representativeness and strictness. Also the gene for DAT is hardly expressed before death, since DAT is an age dependent disease. In the APP gene, four mutations are identified on 717 as 1) Val- Ile 2) Val-Phe 3) Val-Gly 4) 670\Ilys\ Met-Asn \Leu. All these mutations are involvedin autosomal dominant AD. (31) (34)

Investigators are weary of concluding that AD is genetically engineered because ofconflict between Mendel's view on segregation and the patterns of AD. The Mendel pattern does not exist in families of AD patients posing strong limitation to concluding that AD is hereditary. However, the problem with the pattern is that in late onset AD the patient might die of other causes before being diagnosed of

AD. The family history studiesare also limited because of the small sample that is available in studying the condition and also the difficulties in obtaining samples for study. I was expecting the twin studies to strongly agree or disagree with the idea that genetics is the cause of AD. However, that was not the case because obtaining the twins for study is difficult and even when located both of the twins being available for studies is a problem therefore leading to bias of representative samples. Even when the twins are available, one might have AD while the other is only cognitively impaired making the diagnosis of concordance incomplete. (14) (60)

People at Risk

There are several risk factors to AD that include head trauma, family history of Down's syndrome and Parkinson's disease, medical histo ry, psychiatric history, exposuresto environmental and occupational hazards, race and gender. However, the established risk factors are AGE, FAMILY HISTORY OF DEMENTIA and GENETICS. Never the less, most scientists do not believe that these are the only risk factors even though they are the established risk factors. No case control studies have associated Alzheimer's with alcohol, toxic or viral exposures, contact with animals or any other medical history ry. As such, we are left with environmental and genetics as the cause of AD. The epidemiological studies of environmental factors associated with the risk of AD have produced inconsistent and disappointing results to the investigators. However, it is important to remember that while the search for environment causes of AD has been disappointing, many studies have shown an increased risk among those with family historyof AD. This leaves genetic (even though not proven yet) as the cause of AD. (71) (72)

Age is clearly the most accepted of all the risk factors by scientists. The older people become the more likely it is that they will develop AD. In my literature review, it isclear that even so, it can strike people below the age of 50 years though the vast majorities are beyond the age of 65 years of age. The question is why do some people develop AD asthey get older and others do not? Family history of dementia and genetics contributes to AD as people get older. Having a parent, son, daughter and sibling with AD increases ones chances of developing the disease. However, it is important to know that having a relative that has AD does not mean one is going to come down with the disease. If AD is observed in several family members, it could mean that those family members are exposed to some environmental determinants. Another possibility of who is at risk is genetically linked individuals who are observed to show an AD pattern of inheritance. Investigators identify three different chromosomes that are associated with AD and a fourth is being considered for association with AD as well. These chromosomes are 1, 14, 19 and 21. There is no evidence that after the age of 65 years, those

chromosomes are associated withthe majority of the cases of AD cases. The fact that we are unable to avoid aging, choose the family into which we are born, and control our genetic makeup, the risk of one developing AD will always be there. (23)(24)(28)

Future Prospective

The future looks very bright for genetic understanding of AD, its diagnosis and treatments. The fact that investigators have discovered chromosomes 1, 14, 19 and 21 as being associated with AD is not going to stop there. The search will continue and more discoveries will be made especially on gene functions; since it is genes that give messages to body chemicals to act in a certain way. In relationship to AD, it is the gene which givesthe message to cells to die before their time. Just like in cancer cells or tumor cells where the gene message is for the cells to reproduce at a fast rate, causing cells to over multiply. (83)

In the future, it will be important for investigators to develop a diagnostic test that will be accurate for certain forms of AD. If the causes of AD are to be well understood, the genes that are involved and the defects responsible must also be identified. When these genes are identified, then modifications and corrections of the genes will follow. Even though it seems unlikely that an assay for the disease will be developed that is fool proof As we know, there are very few such assays in the field of medicine. Also some investigators have even suggested that onset of AD itself may be inherited and if that is true, may help to explain some of the conflicting results obtained occasionally in relatives with early and late onset of the disease. (83)(85)

Twin studies should continually be looked into because it will always offer many opportunities for understanding the causes and treatment of AD. Even though twins usually share the same environments, they can be separated for the studies and then compared with the twins reared together. In my own opinion, if investigators are to makea huge dent in genetics being the cause of AD, twin cohorts studies must play an important part especially monozygotic twins that show the same genetic make-up as well as being reared together coupled with the same aging time. (14)

Researchers might want to investigate the similarities between genes on chromosome 1 and 14 and the protein they encode for: Also the functions of those genesand their importance to the biology of AD. Researchers should test more people with ADfor chromosome 1 gene defect, and also whether other genes are still at large. Also the sequencing of the new gene is important for screening families as well as for comparison with other genes. (45)(76)

Conclusion

In conclusion, there is growing evidence of genetic causes of AD but this evidence is not conclusive. More research is needed to be definite. After all, it is only recently that late onset AD has been associated with chromosome 19 and also chromosome 1 with early onset AD. The fact that certain chromosomes are associated with the people that have the disease is not enough to conclude that genetics is solely the contributor to AD. The results of my study shed light on the issue of heterogeneity of AD. My literature search findings of the age of the onset of AD as well as segregation analysis suggested that autosomal dominant inheritance does not fit all the cause of an onset of AD before the age of 65. In some studies, evidence of multi-factorial effect as the cause of AD is weak, but still it is worth studying. I believe that the challenge to genetic researchers and epidemiologists in the near future will be untangling the various genetic and non-genetic factors that are associated with AD. (31)

Again the fact that a mutant gene in some families with the early onset is mapped to chromosome 14 and in other families, it is mapped to amyloid processing protein on chromosome 21 shows that familial early onset of AD is genetically heterogeneous. AD affects people of all ethnic backgrounds as well as economic and social backgrounds. As of today, there is no single clinical test to identify AD. Each individual with AD symptoms undergoes a series of diagnostic procedures that includes among many procedures: laboratory tests, PCR tests, computerized tomography, cognitive testing and so on. On the basis of these tests and observations other possibilities are eliminated and the clinician can arrive at the diagnosis for probable and or possible AD. Definite AD diagnosis is usually made at autopsy, since that is the time that the brain is available for thorough examinations. (63) (83) (84)

Bearing in mind that aging is associated with memory loss, it makes it even more difficult to assume or diagnose AD which is a form of dementia, since it is only at death that accurate diagnosis can be made. If genetics is really the cause of AD, the question that keeps bothering me is why this gene(s) is not expressed for 65 or more years after birth. Maybe an understanding of the causes of this delay may help in answering the lingering question of genetics as the cause of AD. If the suppressor gene that is involved in the delay of the Alzheimer's disease gene expression is identified, maybe that will explain the process of the delay of the expression of the AD gene. Also the natural aging process needs to be studied in much detail with genetics as the focal point. The first degree relative of AD should be tested for genetic abnormalities at a younger age. A follow up tests should be done periodically (every ten years) until 65 years of age or more. This should bedone to determine when the gene that codes for AD

becomes activated. These genes obviously have a genetic determinant that may be inactive much earlier in life only to be activated and recognized later in life causing a chronic disease like AD. (77)

Recently, (May 1996) it was found that the majority of women who were taking estrogen medication during their menopause were found not to develop AD when compared with those that did not take the medication. The news reporters also cautioned that the investigators said that more research is needed to confirm these findings. Pursuing information in these possible studies may unlock some of the mysteries of genetic involvement in AD, or at least, unveil further avenues for investigators.

REFERENCES

1) Funk and Wagnalls Corporation; Alzheimer's disease "Microsoft (R) Encarta, Microsoft Corporation. 199 4.

2) Sorbi S. Molecular genetics of Alzheimer's disease; Aging (Milano)(Italy). 48: (6) P 417-425, 1993.

3) Mullan M, Houlden H, Crawford F, Kennedy A, Roques P, Rossor M. Age of onset in familial early onset Alzheimer's disease correlates with genetic etiology. Am J Med. Genet. 48: (3) p. 129- 130, 1993.

4) Nechiporuk A, Fain P, Kort E, Nee Le, Frommelt E, Polinsky RJ, Korenberg Jr., Pulst S.M. Linkage of familial Alzheimer's disease tochromosome 14 in two large early onset pedigrees: effects of marker allele frequencies on lod scores. Am J Med Genet. 48: (1) P.6 3-66, 1993.

5) Corder E.H, Woodburg M.A. Genetic heterogeneity in Alzheimer 'sdisease; a grade of membership analysis. Genetic Epiderniol. 10: (b) p. 495-499, 1993.

6) Faucett C, Ganderman W.J, Thomas D, Ziogas A, Sobel E. Combined segregation and linkage oflate onset Alzheimer's disease

inDuke families using gibbs sampling. Genet Epidemiol. 10: (b) p. 489-494, 1993.

7) Goldin L.R, Gershon E.S. Linkage of Alzheimer's disease to chromosome 19 markers; effect of age of onset assumptions. GenetEpidemid. 10: (b) p. 449-454, 1993

8) Miller L.L, Ploughman L.M, Marazita M.L. Heterogeneity testing forAlzheimer's disease within and between data sets. Genet Epidemiol. 10: (b) p. 443-447, 1993

9) Neuman R.J, Rice J.P, Hampe C.L, Burroughs T.E, Moldin S.O. Linkage analysis of a complex disease: application to familial Alzheimer's disease. Genet Epidemiol. 10: (b) p. 419-424. 1993 .

10) Xu J.f, Taylor E.W, Lung F.W, Chung A s, Chase G.A, Maestri N.E, Meyers D.A The impact of some parameters on linkage analysis of Alzheimer's disease. Genet Epidemiol. 10: (b) p. 407-412,1993.

11) Blossey H, Commenges D, Olson J.M. Linkage analysis of Alzheimer's disease with methods using relative pairs. Genet Epidemiol. 10: (b) p. 377-382, 1993.

12) Bailey-Wilson J.E, Bamba V. Sib-pair linkage analysis of Alzheimer's disease. Genet Epidemiol. 10: (b) p. 371-376, 1

13) Pericak-Vance M.A, St George-Hyslop P.H, Gaskell P.C. Jr., Growdon J, Crain BJ, Hulette C, Gusella J.F, Yamaoka L, Tanzi RE, Roses A D. and others. linkage analysis in familial Alzheimer's : description of the Duke and Boston data set. Genet Epidemiol. 10: (b) p. 361-364, 1993.

14) Bergm AL Heredity in dementia of the Alzheimer's type. ClinGenetJuly 46: (1 spec no) p.144-149, 1994.

15) Van Duijn C.M, Hendriks L, Farrer L.A, Backhovens H, Cruts M, Wehnert A, Holman A, Van Broeckhoven C.A population-based study of familial Alzheimer's disease; linkage of chromosome 14, 19, and 21. Am J Hum Genet. 55: (4) p. 714-727, 1994

16) Mohs RC, Breitner J.C.S, Silverman J.M. and others. Alzheimer's disease: Morbid risk among first degree relatives approximates 50% by90 years of age. Arch Gen Psychiatry. 44: 405-408, 1987.

17) Hughes C.P, Berg L, Domziger W.L. and others. A new clinical scale for the stage of dementia. Brit J Psychiatry. 140: 566-572, 1982.

18) William S. Research and Practice; Practical information for health care Profession: Revolutionizing the Genetics of Alzheimer's disease. Vol. 2 No 2. 1993 .

19) St George-Hyslop P.H, Haines J.L, Farrer L.A, The genetic defect causing familial Alzheimer's disease maps on chromosome 21. science. 235: 885-890, 1987.

20) Tanzi RE, ST. George Hyslop P.H, Haines J.L, The genetic defect ofin familial Alzheimer's disease is not tightly linked to the amyloid beta-protein gene. Nature. 329: 156- 157, 1987.

21) Breitner John C.S. MD PHO clinical genetics and genetic counseling inAlzheimer disease. Annals of internal medicine. 115:

601-606, 1991.

22) Haltia M, Viitanern M, Sulkava R, Ala-Hurula V, Poyhonen M, Goldfarb L, Brown P, Levy E, Houlden H, Crook and others. Chromosome 14 encoded Alzheimer's disease: genetic and clinicopathogical description. Ann Neurol. 36: (3) 335-336, 1994.

23) Alzheimer's association; Advances in Alzheimer's Research News Letter Vol 4, November 1, 1994.

24) Bird T.D, Nemens E.J, and Kukull. Conjugal Alzheimer's disease increased risk in offspring. Annals of Neurology. 34: (3) 396-399, 1993.

25) Mayeux R, Ottman R, Tang MX, Noboa-Bauza L, Marder K, Gurland B Stem Y. Genetic susceptibility and head injury as risk factors for Alzheimer's diesease among community-dwelling elderly persons and their first-degree relatives. Ann Nenrol. 33: (5) 494-501,1993.

26) Amaducci L.A, Fratighioni L, Rocca W.A. Risk factors for clinically diagnosed Alzheimer's diesease: A case control study of an Italian population Neurology. 36: 922-931 1986.

27) Farrer L.A, O'Sullivan D.M, Cupples L.A. Assessment of genetic risk for Alzheimer's disease among first-degree relatives. Ann Neurol.25: 485-493, 1989.

28) Shalat S.L, Seltzer B, Pidcock C. A case-control study of medical and family history and Alzhemer's disease. Am Hum Genet. 147:

(Suppl) A 198, 1990

29) Kennedy AM, Brown J, Rossor M. The genetics of Alzheimer's disease. Clinical Neurology. Vol 3 No 2 1994.

30) Miller M. Will Latest Genetic discovery solve the Alzheimer's disease riddle? National institutes of aging, national institutes of healtp. Communication no 56 page 2 August 1995.

31) Van-Duijn C.M, Ferrer L.A, Cupples L.A, Hofman A. Genetic transmission of Alzheimer's disease among families in a Dutch population based study. J Med Genet. 30 : 640-646, 1993.

32) Pfeiffer E.A. Short portable mental status questionaire for the assesment of organic brian deficit in elderly patients. J Am GeriatrSoc. 23: 433-4 41, 1975.

33) Hachinski V.C, Liff L.D, Zihka E, and others. Cerebral Blood flow indementia. Arch Neural. 32: 632-637, 1975.

34) Hary J. PHD. Alzheimer's disease: clinical molecular genetics. Clinicsin Geriatric Medicine. 10: 239- 247, 1994.

35) Schellenberg G.D , Bird T.D, Wijsman E.M, Orr H.T, Anderson L, Newrnens E, White J.A, Bonnycastle L, Weber J.L, Alonso M.E, Potter H, Henston L.L and Matain G,M. Genetic linkage evidence fora familial Alzheimer's disease locus on chromosome 14. Science . 258: 668-671 , 1992 .

36) Corder E.H, Saunders A M, Strittmatter W.J, and others. Geneticdose of apolipoprotein E, type 4 allele and the risk of Alzheimer's disease in the late onset families.Science. 261: 921-923 , 1993.

37) Hardy J. Framing beta amyloid. Nature Genetics. 1: 233-234 ,

1992.

38) Zeldenrust S.R, Murrel J, Farlow M, Ghetti B, Roses A D, Benson M.RFLP analysis for APP 717 Mutations associated with Alzheimer's disease J Med Genet. 30: 476-478 1993.

39) Bennett C, Crawford F, Osborne A, Diaz P, Hoyne J, Lopez R, Roques P, Duara R, Rossor M, and Mullan M. Eviden that the APOELocus Influences Rate of Disease Progression in late onset familial Alzheimer's Disease but is not causative. Arn J Med Genet. (Neuropsychiatric Genetics) 60: 1-6 , 1995.

40) Frost R. Encircling the mechanism in Alzheimer's Disease . The J. ofNIH Reaseach 4: 48-54, 1992.

41) St George-Hyslop P, Haines J, Rogaey E, Mortilla M, Vaula G, Pencak Vance Mand other. Genetic Locus for a novel familial Alzheimer's disease Locus on Chromosome 14. Nature Genet. 2: 1992

42) Mullan M, Houlden H, Windelspecht M, Fidani L, Lombardi C, Diaz **P**and others. A Locus for familial earl y onsel Alzheimer's disease on the long arm of Chromosome 14 proximal to alpha 1-antichymot rypsin. Nature Genet. 2: 1992.

43) Sisodia S.S, Koo E.H, Beyreuther K, Unterbeck A, and Price D.L, Evidence that Beta-amyloid protein in Alzheimer's disease is not derieved by normal processing. Science. 248: 493, 1990.

44) Strittmatter W.J, Sounders A M, Schineckel D, Perical-Vance M, Enghild J, Salvesen G.S, and Roses A D. Apolipoprotein E high affivity binding to Beta-amyloid increase frequency of type 4 allele in familial Alzheimer's . P roc. Natl. Acad. Sci. 1993.

45) Chartier-Harlin M-C, Crawford F, Houlden H, Warren A, Hughes D, Fidani

L, Goate A, Rossor M, Rognes P, Hardy J. and Mullan M. Early-Onset Alzheimer's disease caused by Mutation at Codon 717 of the Beta-amyloid precursor protein gene. Nature . 353: 844-846, 1991.

46) Burns G. Science of Genetics : An introduction to heredity. FourthEdition page 1-590. 1980 .

47) Breitner J.C.S, Genetic factors in etiology of Alzheimer's disease. In American College of Neuropsychopharmacology: a third generation of progress. Edited by Meltzer H.Y, New York: Raven Press 1987.

48) Heston L.L and White J.A Dementia: A practical guide to Alzheimer's disease and related illness. New York W.H. Freeman and Co. 1983 .

49) Mclhann G. and others. Clinical diagnosis fo Alzheimer's disease report of the NINCDS/ADRDA work group under the autspies of department of health and human Services to ask force on Alzheimer.Neurology. 34: 939-9 44, 1984

50) Nee L.E, and others. Family with Histologicall Confirmed Alzheimer'sdisease Arch, Neurol. 40: 203-208, 1983.

51) Breitner J.C, and Folstein M.F, Familial Alzheimers dementia: A prevalent disorder with specific clinical features. Psycho} Med. 14:63-80 , 1984.

52) Heston L.L, Mastri R L, and Anderson E. Dementia of the Alzheimer;s type: clinical genetics , natural history and associatedconditions. Arch Gen Pschiatry. 38: 1085-1090, 1981.

53) Heyman A. and others . Alzheimer's disease: genetic aspects andassociated clinical disorders. Ann Neurol. 14: 507-515 , 1983.

54) Mohs RC, Breitner J.C.S, Silverman J.M, Davis K.L, Alzheimer's disease: Morbid risk among first-degree relatives approximates 50% byninety years of age. Arch Gen Psychiatry. 44: 405-408, 1987.

55) Martin R L, Gerteis G, Gabrielli F Jr. A Family-Genetic study of dementia of

Alzheimer type. Arch Gen Psychiatry. 45: 894-90 0, 1988.

56) Namba Y, Tomonaga M, Kawasaki H, Otoma E and Ikeda K. Apolipoprotein E. immunoreactivity in Cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's diesease and Kuru plaque amyloid in Grentzfeldt-Jakob disease. Brain Research. 541: 163-166 ,1991.

57) Murakami M, ushio Y, Ohta T, Morino Y, and others , Immunochemical localization of apolipoprotein E in the human glialneoplasms. J Clin Invest. 82: 177-188, 1988.

58) Silverman J.M, Breitner J.C.S, Mohs R C, and Davis K, Reliability of the family history method in genetic studies of Alzheimer's disease andrelated dementia. Am J Psychiatry 143: 1279-1 282, 1986

59) Gershon E.S, Guroff J.J, Information from relatives: diagnosis ofaffective disorders. Arch Gen Psychiatry. 41: 173-180, 1984.

60) Breitner J.C.S, Gatz M, Bergem A.L.M, Christian J.C, zmortimer J.A, McClean G.E, Heston L.L, Welsh K.A, Anthony J.C, Folstein M.F, and Radebough T.S. Use of twin Cohorts for research in Alzheimer's disease. Neu rology. 43: 261-267, 1993.

61) Lykken D.T, McGue M, Tellegan A. Recruitment bias in twin research: the rule of two-thirds reconsidered. Behav . Genet. 17: 343362 1987.

62) Breitner J.C.S. Longitudinal tudies of twins with Alzeimer disease: preliminary findings. (abstract) Gerontologist 31: 139, 1991.

63) Richter R.W, Blass J.P, Alzheimer's disease: a guide to praticalmanagment Part IV. Pages 159-167, 1994.

64) Martin R.L, the genetics of Alzheimer disease: Indentification of persons at high risk. Alzheimer's disease: Current Research in early diagnosis edited by Robert E. Becker and Ezio Giacobini. Taylor andFrancis New York Inc. Pages

31-48, 1990.

65) Heston L.L, Genetic counselling and the presenile dementidas, SocBiol. 23: 135-141, 1976.

66) Olson M.L, Hood L, Cantor C, and Botstein D. A common languag for physical mapping of the human genone. Science 245: 1434-1435, 1989.

67) Wizniewski H. Molecular genetic markers in familial Alzheimer's therapy early diagnosis, editions R e. Becker and E Giacobini NewYork: Taylor and Francis 1990

68) Van-Broeckhoven C, Backhoven H, Cruts M, Martin J.J, Crook R, Houlden H, and Hardy J. APOE genotype does not modulate age ofonset in families with chromosome 14 encoded Alzheimer's disease. Neuroscience letters 169: 179-180, 1994.

69) Kilpatrick C, Burns R, Blumbergs P. Identical twins with Alzheimer's disease. Journal of Neurology, Neurosugery and Psychiatry. 46 : 42- 425, 1983.

70) Cook R.H. Schneck S.A and Clark B.B. Twins with Alzheimer'sdisease. Arch Neurol. 38: 300-301, 1981.

71) Breitner J.C.S, Murphy E.A, Folstein M.F, and Magruder-Habib K.Twin studies of Alzheimer's disease: an approach to etiology and prevention. Neurology of Aging. 11: 641-648, 1990.

72) Broe G.A, Henderson AS, Creasey H Mccusker E, Korten AE, formAF, Longley W, and Anthony J.C. A Case-control study of Alzheimer's disease in Australia. Neu rology. 40: 1698-1707, 1990.

73) Anderson N.C, Endicoot J, Spitzer R.L, Winokur G. The family history method using diagnostic criteria. Arch Gen Psychiat ry. 34: 1229-1235 , 1977.

74) Crawford F, Hardy J, Mullan M, Goate A, Hughes D, Fidani L, Roques P, Rossor M, Chartier-Harlin M-C. Sequencing of exons 16 and 17 of the Beta-amyloid precursor protein gene in 14 families withearly onset Alzheimer's

disease fail to reveal mutations in the Beta- amyloid Sequence . Neuroscience letters. 133: 1-2, 1991.

75) Chartier-Harlin M-C, Crawford F, Hamandi K, Mullan M, Goate A, Hardy J, Backhovens H, Martin J.J, Van-Broekhoven C. Screening for the Beta amyloid precausor protein mutation (APP 717: Val>ILE) in extended pedigrees with early onset Alzeimer's disease. Neuroscience letters 129: 134-135, 1991.

76) Levy-Lahad E, Wijsman E.M, Nemens E, Anderson L, Goddsd K.A.B, Weber J.L, Bird T.D, Schellenberg . A familial Alzheimer'sdisease locus on chromosome 1. Science 269: 970-976, 1995.

77) Albert M.L and Knoefel E. Clinical Neurology of aging. Second Edition, New York Oxford University press pages 136-149, 1994.

78) Owen M.J, James L.A, Hardy J.A, Williamson T. and Goate A M. physical mapping around the Alzheimer's locus on the proximal longArm of Chromosome 21. Am. J. Hum Genet. 46: 316-322, 1990.

79) Goate A, Chartier-Harlin M-C, Mullan M, Brown J, Crawford F, FidaniL, and others . Segregtion of a missense mutation in the amyloid precursor protein gene with familial Alzheimer’s disease. Nature 349: 704-708, 1991.

80) Kambob M.I, Sangbera D.K, Ferrel R E, and Dekosky S.T. APOE 4 associated Alzheimer's disease risk is modified by Alpha 1-antichemotrypsin polymorphism. Communication. 47: 2-4, 1995.

81) Liddlell M, Williams J, Bayer A, Kaiser F, Owen M. Comfirmation of association between the E4 allele of apolipoprotein E and Alzheimer's disease. J med Genet. 31: 197-200, 1994.

82) Brousseau T, Legrain S, Berr C Gourlet V, Vidal O and Amouyet P. Confirmation of the E4 allele of the apolipoprotein E gene as a risk factor for late-onset Alzheimer's disease. Neu rology. 44: 342-344, 1994.

83) Nee L, E. Dealing with the genetic aspects of Alzheier's disease.Chapter 2 Pages 169-177, 1992.

84) Richter R. W, Blass J. P. Projetion for the furture. Chapter 4Pages 195-201, 1992.

85) Li Ge, Silverman J.M. and Mohs R. C. Clinical genetic studies of Alzheimer's disease. The Psychiatric Clinics of North America. vol 14 (2) June 1991.

86) Levy-Lahad E, Wasco W, Poorkaj P, Romano D.M , Oshima J, Pettingell W.H, Yu c-e, Jondro P.D. and others. Canditate gene for chromosome 1 familial Alzheimer's disease locus. Science. 296: 973-977 , 1995.

87) Levy-Lahad E, Wijsman E.M, Newmens E, Anderson L, Goddard K.A.B, Weber J.L, Bird T.D, Schellenberg G.D. A Familial Alzeimer's disease locus on chromosome 1. Science. 269: 970- 973,1995.

88) Anderson B, Fallen D, The Genetic of Alzheimer's disease: New Cluesgenerate optimism. Alzheimer's Association, July 1995.

89) Tomlinson B. E, Plaques, tangles and alzheimer's disease. PhycholMed. 12: 449-459, 1982.

90) Boller F, Forette F, Khachaturian Z, Poncet M, and Christen Y. Heterogeneity of Alzheimer's disease. Springer-Verlag Berlin Heidelberg,New York, pages 81-129, 1992.

91) Goodman R. M and Gorlin R. J. Atlas of the face in geneticdisorders. Second Edition Pages 25-34.

92) Larsson T, Jacobsen G, Senile dementia. Acta Psychiatr . Scand .Suppl. 167: 1-159, 1963.

93) Sjogren T, Sjogren H, Lindgren A.G .H. Morbus Alzheimer andMorbus pick: A genetic, clinical and patho-anatomical study. ActaPsychiatr. Neuro.

Scand. Suppl. 82: 1-153, 1952.

94) Chandra V, Phillipose V, Bell P.A. and others Case control study oflate onset probable Alzheimer's disease. Neurology 37: 1295-1300, 1987.

95) Kallman F. Genetic aspects of mental disorders of later life Kaplan 0 edition Mental Disorders in later life, Stanford University Press.Pages 26-46, 1956.

96) Jarvik I, Ruth V and Matsuyama S. Organic brain syndrome and aging. A six-year follow up of surviving twins. Archieves of General Psychiatry37: 280-286, 1980.