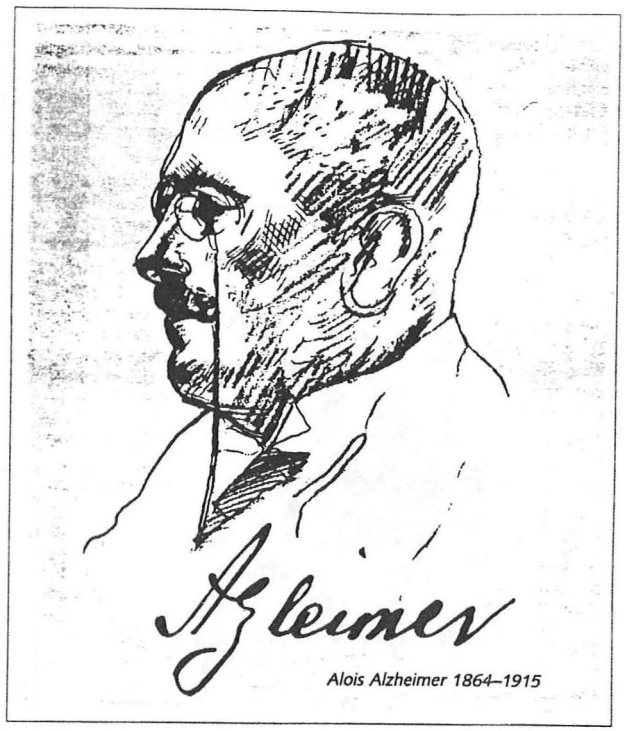

Genetic Basis of Alzheimer's Disease: Death From Cerebral Indigestion

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In 1907 Alois Alzheimer, the German neurologist, described the first case of the disease that bears his name (Alzheimer, 1907). Alzheimer studied the brain of a 51 year old woman who died with presenile dementia. He took advantage of newly available silver stains to visualize the two pathologic hallmarks of the disease, namely, the amyloid plaques and the neurofibrillary tangles. He believed that these two lesions were responsible for the atrophy and progressive loss of function in the brain.

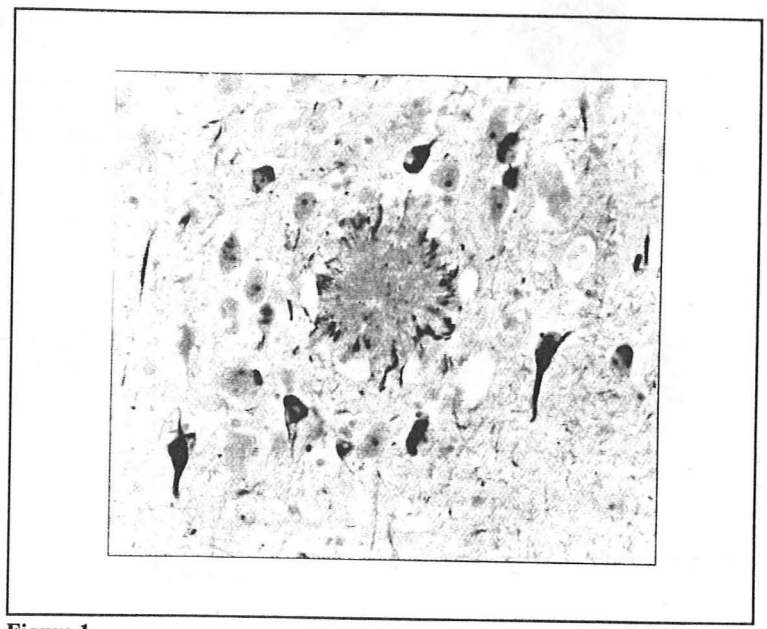

Figure 1.

Figure 1 shows a modern version of the senile plaque of Alzheimer's disease (Selkoe, 1995). It is a dense collection of fibrillar material that stains a homogenous pink with certain dyes. This structure shows classic green birefringence when stained with Congo red and seen in polarized light. Also seen in this micrograph are neurons that stain darkly with silver stains owing to tangles of microtubules within the cytoplasm. These are called neurofibrillary tangles. The amyloid plaques and the neurofibrillary tangles are the hallmarks of Alzheimer's disease.

Figure 2 shows the proposed pathway for the evolution of the senile plaque as deduced from examination of brains from subjects with Down's syndrome, all of whom develop Alzheimer's disease if they live long enough. The earliest form of the plaque, shown in a and b, consists of a diffuse deposit of amyloid proteins. At this stage there is no accompanying cellular degeneration. With time the plaque condenses and it develops a dense core as shown in panels c and d. These plaques are called "neuritic" because they frequently contain trapped neurons within them.

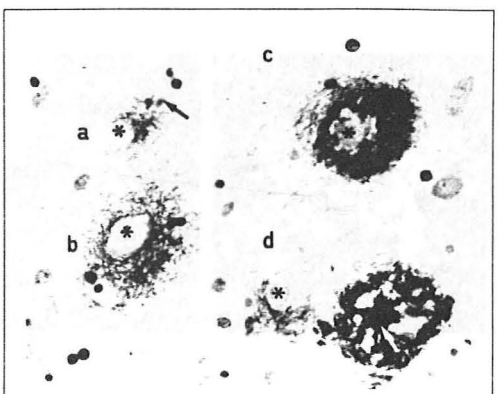

Fig. 2. Plaques from cases of Down syndrome, stained by a sensitive methenamine silver technique with Nissl counterstain. Note that amyloid or 'pre-amyloid' deposits in diffuse plaques **(a, b, c)** *are frequently seen to accumulate around neuronal cell bodies* **(asterisks)** *and dendrites, but show no obvious relationship with capillary blood vessels* **(black arrow)**. *These photographs show a possible progression of stages, the* **white arrow** *in* **d** *indicating the dense amyloid core of a fully-developed plaque.* Taken from Allsop, D. *et al.* (1989) *Neuropathol. Appl. Neurobiol.* 15, 531–542, with permission.

Figure 2.

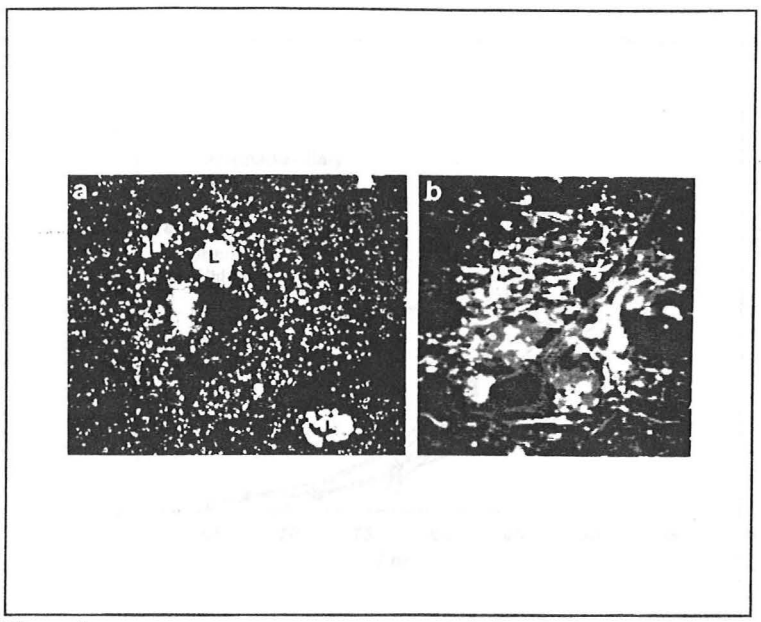

Figure 3.

Figure 3, b shows a neuritic plaque with a trapped neuron (Terry, 1994). In panel a the same brain has been stained with an antibody against synaptophysin, which identifies synapses. The large clear areas devoid of synapses surround the amyloid plaques, and they also occur in regions where no plaques are visible. This loss of synapses correlates best with the degree of dementia in Alzheimer's disease (Terry, 1991). It is paradoxical that synapses are lost in regions where no plaques are visible. We will return to this point later.

Figure 4 shows the incidence of Alzheimer's disease in six different populations as a function of age (Katzman, 1994). You can see the remarkable increase as individuals exceed the age of 70. By age 85 40% of people in certain populations will have clinically diagnosed Alzheimer's disease. A figure this high has recently been reported from a survey in Boston (Evans, 1989). Thus, Alzheimer's disease appears to have a high incidence in all racial and ethnic groups as a function of age. The cases occurring before age 65 are called pre-senile dementia. The ones occurring after this age are often called senile dementia. As we will see, all Alzheimer's disease, whether it occurs early or late, has the same pathologic mechanism.

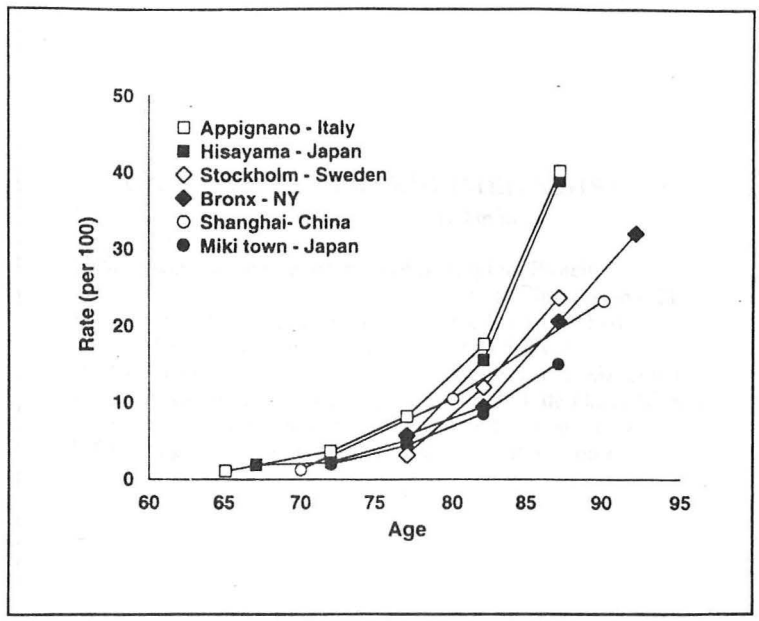

Figure 4.

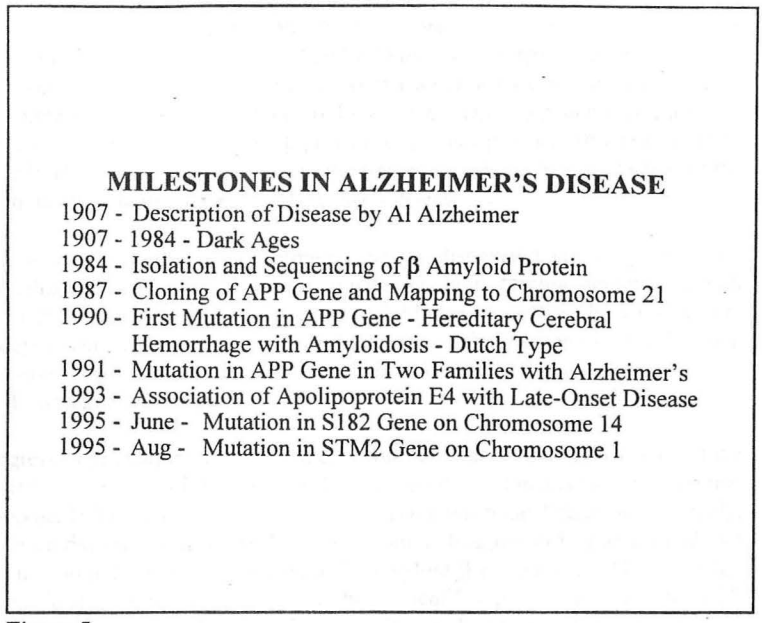

Figure 5.

Figure 5 lists the milestones in Alzheimer's disease research, which will form the basis of today's lecture. You can see that the pace of discovery for this field is rising geometrically. As I've already mentioned, the disease was described by Alzheimer in 1907 (Alzheimer, 1907). Unfortunately, his conclusions were not universally accepted. Recall that this was the age of bacteria. Most physicians felt that Alzheimer's disease was a manifestation of syphilis (Thomas, 1987). It was also the age of Freud. Psychiatrists believed that Alzheimer's dementia was functional and secondary to psychosis. In the 1950's another confusion developed. The theory arose that Alzheimer's disease was a product of arteriosclerosis and the term "hardening of the arteries" was coined. In fact, arteriosclerosis is a relatively uncommon cause of dementia in the elderly and Alzheimer's disease is much more frequent (Joachim, 1988).

The 74 year period from 1907 to 1984 can therefore be called the "Dark Ages." This era ended in 1984 when Glenner isolated the protein called β-amyloid protein that forms the core of the amyloid plaque (Glenner, 1984). Glenner did this isolation by taking advantage of the near indestructibility of the amyloid complex. When isolated amyloid plaques were treated with crude collagenase, only the β-amyloid protein survived, and this allowed its purification and sequencing. The β-amyloid protein had a molecular weight of about 4000 daltons and was composed of approximately 40 amino acids. This was the fundamental breakthrough that opened the field. Glenner, who was the first person to identify the underlying cause of all amyloidosis, died recently before he could be honored sufficiently for his revolutionary achievements.

Three years later in 1987, several groups simultaneously cloned the gene for the amyloid protein and mapped it to chromosome 21 (Kang, 1987; Selkoe, 1994). The gene encoded a precursor of the amyloid β protein and so it was called the Amyloid Precursor Protein gene or APP. This mapping immediately stimulated the field. It is well known that all Down's syndrome patients will develop Alzheimer's disease if they live long enough (Oliver, 1986). Down's patients have an extra copy of chromosome 21 and therefore the amyloid β protein gene. They produce 50% more amyloid protein that normal individuals. This correlation was the first evidence that overproduction of the amyloid protein might be the initiating event in Alzheimer's disease.

In 1990 the first mutation in the amyloid protein gene was discovered, not in a patient with classical Alzheimer's disease, but in a Dutch individual with hereditary cerebral hemorrhage with amyloidosis (Levy, 1990). These individuals have a few amyloid plaques, but they have much more prominent amyloid deposits in microvessels of the brain leading to cerebral hemorrhage (vanDuinen, 1987). This is an autosomal dominant disease and affected individuals were shown to have an amino acid substitution in the amyloid protein gene.

The next progress came from the study of patients with familial Alzheimer's disease of the classic type. About 10% of the cases of Alzheimer's disease are inherited in a clear-cut autosomal dominant pattern (Selkoe, 1994). The families are easily detected when the disease appears early, i.e., before the incidence rises in the aged. In 1991 Goate and colleagues in England found two early-onset families in whom the defect was linked to the amyloid β protein gene (Goate, 1991). By sequencing the gene in affected relatives, they were able to identify a point mutation in the APP gene that caused Alzheimer's disease in these two families. This finding firmly linked the amyloid protein gene to the pathogenesis of Alzheimer's disease.

In 1993 a surprising development occurred. Patients with late-onset Alzheimer's disease were found to have a very high frequency of the ϵ4 variant of the gene encoding apoE, a secreted protein of the lipoprotein transport system (Strittmatter, 1993). This remarkable finding has substantial implications that I will describe in detail. Finally, last summer two papers appeared that describe mutations in a gene on chromosome 14 and another on chromosome 1 that produce early-onset autosomal dominant Alzheimer's disease (Sherrington, 1995; Levy-Lahad, 1995). Although these mutations are not in the APP gene itself, they nevertheless lead to the deposition of amyloid β protein, and they produce dementia as early as age 40. The genes on chromosome 14 and 1 produce proteins that are highly related to each other. In the remainder of the talk, I will focus on what we have learned from these milestones.

Now let's see what the β-amyloid protein looks like (Figure 6). The precursor of the β-amyloid protein is a long polypeptide chain that sits on the outer membrane of the cell. The protein is not only present on neurons. It is present on most other cells in the nervous system including astrocytes and microglia. It is also present on nearly every other cell in the body. This is one of the biggest surprises of Alzheimer's disease, namely, that the protein at fault is not a specific product of the brain, but rather is produced in all organs.

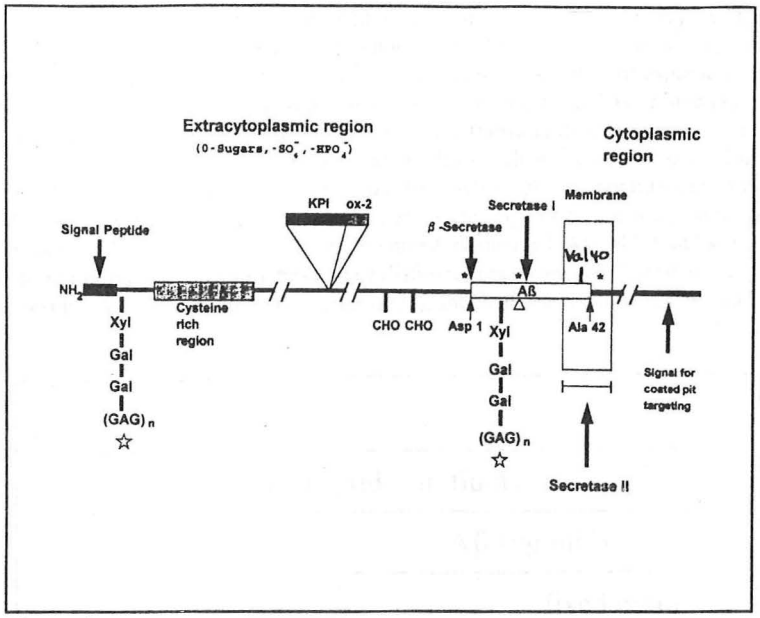

Figure 6.

The β-amyloid precursor protein has a signal sequence at the amino terminus that directs it to the plasma membrane. It also has a hydrophobic sequence that anchors it in the plasma membrane and forms a transmembrane domain. There is a short sequence that projects into the cytoplasm of the cell. The precursor protein is synthesized in two major forms owing to alternative splicing of the messenger RNA. Some copies of the protein contain a short sequence of amino acids that is an active protease inhibitor of the protease nexin type. Other copies of the protein lack this protease inhibitor insert. The external domain contains a variety of modifications including heparin-like glycosaminoglycan carbohydrate chains indicated by the star. It also contains sulfate and phosphate residues whose positions have not been identified.

The amyloid precursor protein is processed proteolytically in a variety of ways. Under normal circumstances, much of the protein is cleaved before it reaches the cell surface. Cleavage is mediated by a protease called secretase-1 which is also known as α-secretase. This releases a soluble fragment that is secreted into the extracellular fluid. Notice that secretase-1 cuts within the fragment that has the potential to form the amyloid deposit. If secretase-1 cleaves the precursor, then the protein can never form amyloid deposits.

If secretase-1 does not cleave the precursor, then the protein is susceptible to cleavage by another protease designated β-secretase. Cleavage at this point generates the amino terminus of the fragment that forms amyloid fibrils. This is called the amyloid β fragment or Aβ. The aspartic acid at this point is considered to be residue 1 of the Aβ peptide. Once this cleavage has been made, a second protease called secretase-2 cleaves the β protein within the transmembrane segment. This protease can cleave at one of several sites. Under normal conditions cleavage occurs at a valine residue to generate a 40-residue peptide, designated Aβ1-40. Although this peptide can form amyloid fibrils it does so only rarely. A much more dangerous cleavage occurs at the alanine at position 42 of the sequence. This produces a 42 residue fragment, designated Aβ1-42, that has the highest potential to form fibrillar amyloid structures. The distinction between whether secretase-2 cuts the protein at residue 40 or 42 is the major determining factor in early-onset Alzheimer's disease.

A surprising finding is that the 1-40 βAP fragment is a normal constituent of cerebrospinal fluid and plasma (Figure 7) (Seubert, 1992). Moreover, it is produced by mixed cultures of brain cells (Figure 7) and by cultured fibroblasts.

Aβ peptides in fluids

		Aβ (ng ml^{-1})	
Source	CSF	Plasma	Mixed brain cell culture
Human	2.5	0.9-±0.4	4.0
Dog	2.0	2.0	
Guinea-pig	2.5	4.5	
Rat	1.5	ND	

Figure 7.

Figure 8 shows the various mutations in the APP gene that give rise to Alzheimer's disease. Notice that all of these are point mutations that produce amino acid substitutions. A single copy of one of these mutant proteins is sufficient to cause disease in a dominant fashion. These findings indicate that the pathology of Alzheimer's disease is caused by a gain-of-function mutant protein and not the loss of function of the normal protein.

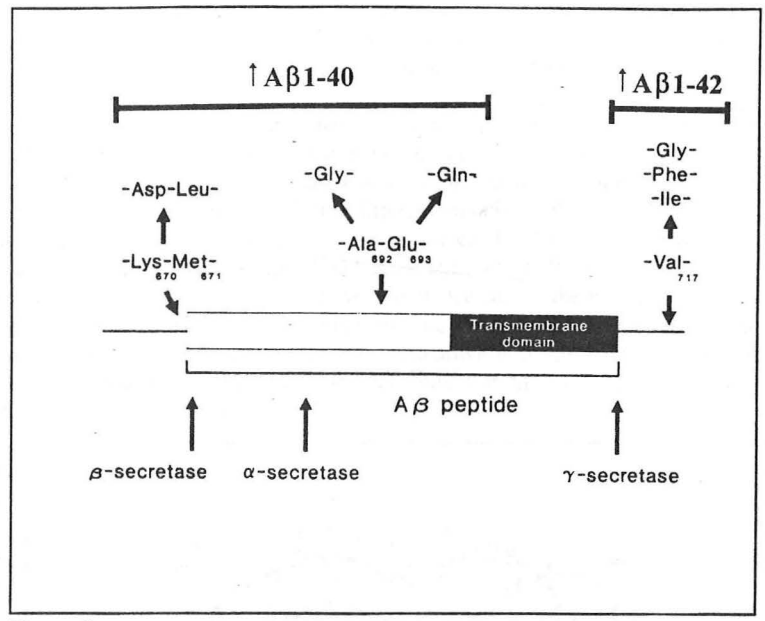

Figure 8.

The effects of APP mutations on the protection of amyloid have been studied in two ways. First, genes encoding the wild-type or mutant forms of APP have been introduced into normal cells in tissue culture through DNA transfection techniques. The amounts of Aβ1-40 and Aβ1-42 have been measured. Second, cultured fibroblasts from affected patients have been compared with normals. Normal fibroblasts produce small amounts of Aβ1-40 (Haass, 1992). The production of Aβ peptides by the mutant fibroblasts is then compared to that produced by the normal cells.

From Figure 8, you will note that all of the mutations that cause Alzheimer's disease occur in or around the Aβ protein sequence. The tissue culture studies reveal that they have different effects. The first mutations to be discovered were the alanine at 692 changed to glycine and the glutamic acid at 693 changed to glutamine (Selkoe, 1994). These two different mutations occur in patients with the Dutch type of hereditary cerebral hemorrhage with amyloidosis. They both increase the production of the Aβ1-40 fragment. The second class of mutations, shown on the left is a double mutation that was originally found in several Swedish families (Citron, 1992). This mutation changes two amino acids, Lys-Met to Asp-Leu. These two amino acids are immediately

adjacent to the site of cleavage by the β-secretase that gives rise to the Aβ peptide. Apparently, these mutation increase the initial cleavage by the β-secretase (Citron, 1992, 1994; Cai, 1993). The second site of cleavage occurs at the "normal" position to produce Aβ1-40. The net result is a massive increase in production o f the Aβ1-40 fragment (Citron, 1992, 1994).

The final set of mutations occurs on the other side of the Aβ sequence. These mutations occur at residue 717 which is somewhat downstream from the site of cleavage by the γ-secretase (Goate, 1991; Karlinsky, 1992). (Numbering is relative to the sequence of the spliced form of APP which contains the protease inhibitor sequence.) Three different mutations, all at the same residue, have been found in different families (Selkoe, 1994). The valine can be changed to an isoleucine, a phenylalanine or a glycine. In each case the mutation has the same affect. It does not increase the total amount of β peptide formed. Instead, it changes the site of cleavage by the γ-secretase so that the Aβ1-42 fragment is formed instead of the 1-40 fragment (Suzuki, et. al., 1994). As I mentioned earlier, the 1-42 fragment has a much higher tendency to form amyloid fibrils and therefore these patients develop Alzheimer's disease even though the total amount of Aβ protein is normal. It is simply the proportion of Aβ1-42 that is increased.

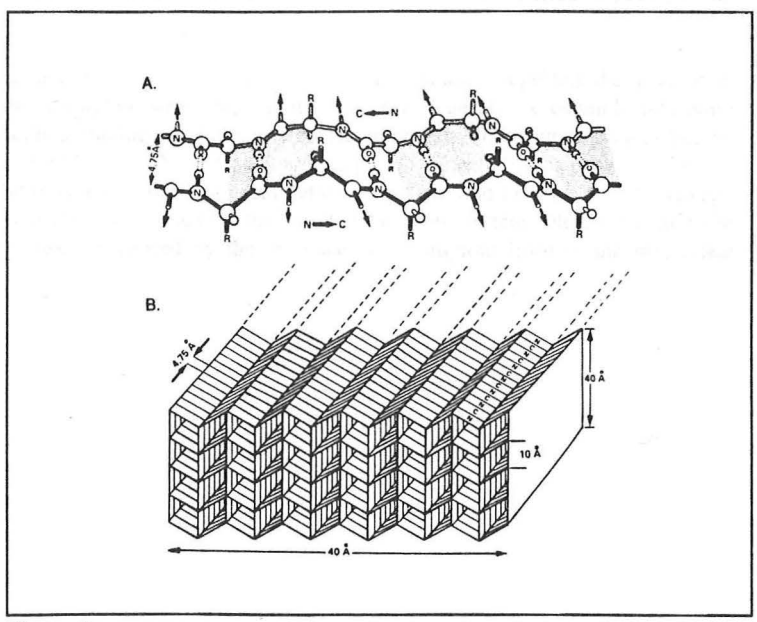

Figure 9.

In order to understand the mechanism by which amyloid peptides cause this disease, we first have to understand the mechanism of amyloid fibril formation. Amyloid peptides not only cause Alzheimer's disease. Other proteins in addition to Aβ1-42 can form amyloid structures, and they all cause disease in the tissues in which they are deposited (Sipe, 1992). All amyloid proteins appear to have a similar structure (Benson, 1992). Figure 9 shows the general structure of all amyloid fibrils that have been isolated to date. They are all polymers of proteins that pack together in a confirmation known as the "β-pleated

sheet." The β-pleated sheet configuration is found in the interior of many proteins where they pack together with other elements of the peptide chain. When these sheets occur on the exposed surface of proteins they have the capacity to pack together with adjacent protein molecules to form long, repeating polymers. In the amyloid proteins these molecules are packed in an anti-parallel orientation: the amino terminus of one peptide chain is adjacent to the carboxy terminus of the next peptide chain. As polymerization progresses, the chains begin to stack up to form twisted helices. One characteristic of amyloid chains is that they all bind the dye Congo red in a particular orientation that is related to the repeating structure of the polymer. Because all of the dye molecules are in the same orientation they have the ability to refract light. When examined under a polarizing microscope this gives the classic green birefringence that is characteristic of amyloid.

Many proteins are able to form amyloid polymers, and these produce a variety of diseases. Amyloid fibrils form under one of two conditions. The first is expression of very high levels of a normal protein such as occurs when immunoglobulin-light chains are overproduced to form the amyloidosis of multiple myeloma (Bentson, 1995). A similar phenomenon occurs in the pancreas where high concentrations of the protein amylin form amyloid plaques in the islets of patients with Type 2 diabetes.

The second cause of amyloid precipitation is a point mutation in a protein that gives it an enhanced ability to form amyloid structures (Bentson, 1995). The classic example is a point mutation in transthyretin, a binding protein that circulates in plasma. The mutant protein has the propensity to form amyloid fibrils. These deposit around peripheral neurons, causing a peripheral neuropathy. Neuropathy is not unique to transthyretin. Many amyloid proteins tend to damage neurons either in the central nervous system or the peripheral nervous system. Neurons seem to be especially sensitive to toxicity caused by the presence of an amyloid fibril in the immediate neighborhood.

The kinetics of amyloid fibril formation are illustrated in Figure 10 (Jarrett and Lansbury, 1993). The first step is the formation of dimers and higher order linear aggregates. This process is relatively slow because each step is reversible and the overall rate is dictated by the equilibrium constants for the addition of each monomer. However, once the fibril has grown sufficiently to form a helical structure all of this changes. Now the newly added monomers form multiple contacts with multiple layers of the existing chains. This process is no longer linear. The dissociation rate of the monomers in markedly reduced and the fibril grows explosively. A characteristic of this process is the lag phase. When monomers are mixed together in vitro, one does not initially see the formation of fibrils. This formation occurs only after a lag phase during which the monomers are assembling to form the first helical structure. This process is referred to as nucleation. Once nucleation is reached, the further growth of the fibril is explosive. Moreover, because of the multiple contacts these fibrils are nearly impossible to dissolve. They resist detergents and they also resist proteases. Indeed, Stanley Pruisner was able to purify the prion amyloid fibril by digesting brains with powerful proteases and isolating the prion fibrils, which were the only proteins in the brain that could resist this treatment.

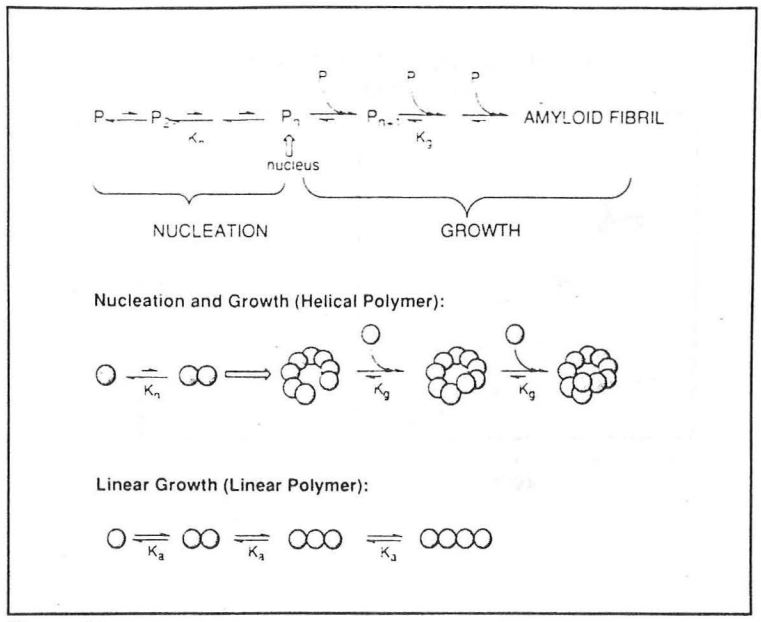

Figure 10.

The same difficulty that scientists have in digesting amyloid fibrils is also faced by the body. Once the stage of nucleation has been reached and the fibrils adopts their final structure, these proteins are indigestible. There is no evidence that the body can destroy them (Selkoe, 1994). However, the body is probably able to destroy the small aggregates that form during the nucleation phase. If these aggregates can be destroyed before they form a nucleus, then the amyloid fibril cannot form.

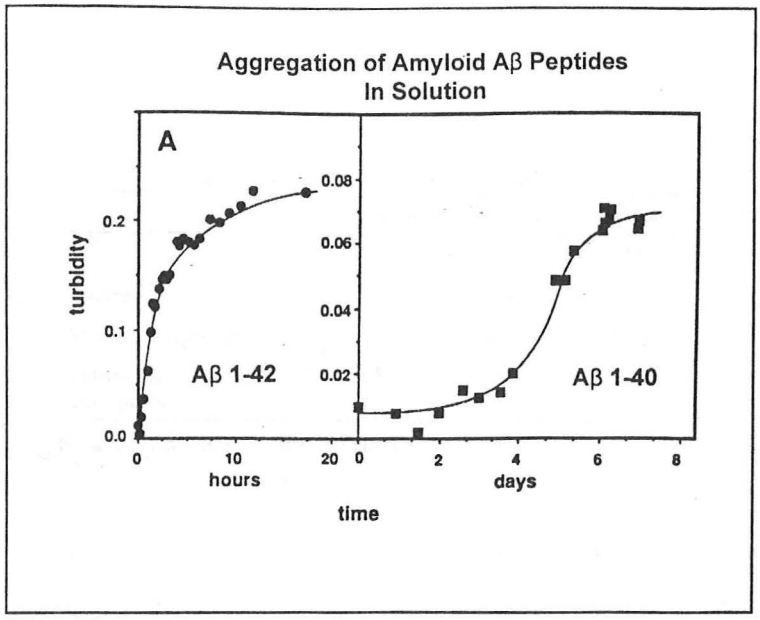

Figure 11.

Figure 11 illustrates the importance of this phenomenon for Alzheimer's disease. Lansbury measured the formation of amyloid fibrils in vitro by measuring the turbidity of a solution (Lansbury, 1995). On the right you see the results when they used the Aβ1-40 peptide. There was a lag of four days in order for nucleation to occur. Thereafter, polymerization was very rapid. In striking contrast, the Aβ1-42 peptide, shown on the left, formed amyloid fibrils within minutes. There was no lag phase. These findings indicate the difference that only two amino acids can make. The Aβ1-42 peptide nucleates so rapidly that a lag is hardly seen. These in vitro findings would suggest that the Aβ1-42 peptide can be much more dangerous than the Aβ1-40 peptide in brains of patients. There is no time for the body to destroy the pre-nuclear phase.

Once the nucleus of the fibril is formed by Aβ1-42, then Aβ1-40 can polymerize with it as fast as Aβ1-42 (Lansbury, 1995). Small amounts of Aβ1-42 can therefore seed the deposition of large amounts of Aβ1-40.

The clinical relevance of these findings is illustrated in a recent study (Gravina, 1995) (Figure 11a). They ground up whole brains of individuals who died of Alzheimer's dementia and they measured the absolute amounts of the Aβ1-40 and the Aβ1-42 peptides. Control brains had no detectible amounts of either peptide. Brains from patients with relatively "pure" Alzheimer's disease which had lots of plaques and tangles but little vascular involvement, had a massive elevation in the Aβ1-42 peptide in the amyloid plaque. There was relatively little of the Aβ1-40. The amount of Aβ1-42 was elevated to the same extent in patients who had substantial amyloid deposits in the microvessels of the brain. However, in these brains the amount of Aβ1-40 was also elevated.

Amounts of Aβ Peptide In Whole Brain

	No.	Aβ1-40	Aβ1-42	Aβ1-42
		pmol/g		%
Control		< 7	< 7	
Minimal angiopathy	9	32	714	96%
Substantial angiopathy	6	5001	826	24%

From Gravina, et. al., J. Biol. Chem. 270, 7013, 1995

Figure 11a.

These data are consistent with an emerging model which says that the Aβ1-42 peptide is responsible for the amyloid plaque, whereas the Aβ1-40 peptide may have a higher tendency to precipitate in blood vessels, producing a more vascular type of Alzheimer's disease. The trouble with the Gravina study is that the patients were not classified as to the cause of Alzheimer's disease. Presumably, these were elderly individuals with late-onset type of disease related to apoE4 (see below).

A variety of in vitro studies have shown that aggregated Aβ peptides are toxic to neurons in tissue culture (reviewed in Selkoe, 1994). The sole requirement is that the proteins be aggregated in amyloid fibrils. It doesn't matter whether the aggregates are formed form Aβ1-40 or Aβ1-42. Once the aggregates form, they are toxic to neurons. Figure 12 shows an example of the progressive killing of neurons that have been presented with an amyloid filament composed of Aβ fibrils.

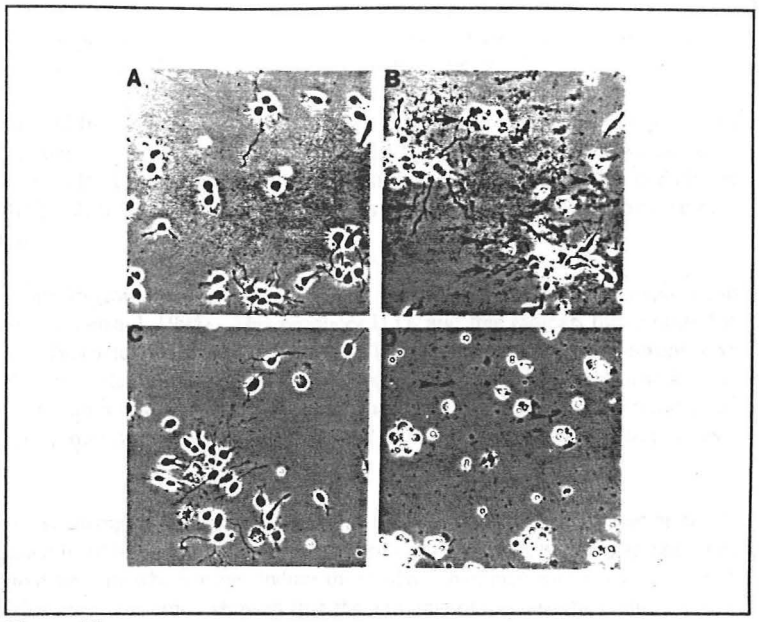

Figure 12.

David Schubert and his colleagues have performed a fascinating series of studies designed to elucidate the mechanism by which these insoluble fibrils kill neurons (Behl, 1994; Schubert, 1995). These investigators have shown that the presence of Aβ fibrils causes neurons to produce large amounts of hydrogen peroxide, a substance that is known to be toxic. The hydrogen peroxide may be produced by an NADPH oxidase of the flavin type because production can be blocked by known inhibitors of this class of enzymes. When H_2O_2 production is blocked, toxicity does not occur. Toxicity can also be eliminated by destruction of the hydrogen peroxide with catalase, or by the scavenging of free radicals with antioxidants such as Vitamin E.

Hypothesis: Alzheimer's As A Digestive Disease

I consider Schubert's findings to be extremely interesting. The limitation, of course, is that they have only been performed in the environment of isolated tissue culture. Nevertheless, they raise interesting questions, and lead to an interesting hypothesis.

How do amyloid fibrils stimulate the NADPH oxidase? Schubert seems to believe that this is a toxic effect owing to perturbation of membrane lipids (Schubert, 1995). It would be far more interesting if this effect were regulatory. Is it possible that neurons and other cells in the brain "intentionally" produce hydrogen peroxide when faced with an indigestible substance such as an amyloid fibril?

Neutrophils are known to have a classic NADPH oxidase that is induced by exposure to bacteria (reviewed in Forehand, 1995). This produces H_2O_2 and free radicals that modify the proteins and lipids of the bacteria so that the organism is killed. Is it possible that neurons have a related NADPH oxidase that is induced by amyloid proteins and is intended to produce free radicals that oxidize the amino acids of the β-protein so as to facilitate its ultimate degradation? Oxidatively damaged proteins are known to be degraded more readily by a variety of proteases (Forehand, 1995).

If the neuron is attempting to digest the amyloid, then it would make sense that proteases should be upregulated in addition to oxidases. As far as I can determine, no one has examined neuronal cultures to determine whether they induce the production of proteases when faced with amyloid fibrils. However, one study showed that the amounts of the enzyme cathepsin D, a lysosomal protease, are increased in Alzheimer's brains (Diedrich, 1991).

All of these considerations lead me to suggest the following purely hypothetical scenario. In Alzheimer's disease the increased formation of amyloidogenic peptides is sensed by neurons, and perhaps other cells in the central nervous system. In response, the cells activate NADPH oxidases and produce increased amounts of proteases in an attempt to destroy the amyloidogenic protein. If these mechanisms act soon enough, i.e., before the protein has nucleated to form an indigestible fibril, then they may be successful. The cells may digest the pre-amyloid proteins, thereby preventing amyloidosis, but at a terrible price. As an innocent bystander crucial components of the neurons, including membranes and microtubules, are damaged. Because of the long lifespan of the neuron this damage is cumulative. Eventually, the neuron dies.

Death of the neuron has two consequences: 1) it decreases the local production of amyloid β protein by removing one source; and 2) it removes one source of the oxidases and proteases that are the major protection against amyloid plaque formation. Whether or not a neuritic plaque forms at that site is dictated by the balance of amyloidogenic as opposed to protective effects of neuronal death. This scenario would explain the lack of correlation between the sites of amyloid plaques and the extent of neuronal damage in particular areas of the brain.

It should be possible to test this hypothesis by measuring the activity of oxidases and proteases in the brains of mice that have been genetically engineered to develop Alzheimer's disease.

Very recently, mutations in two additional genes were found to cause early-onset Alzheimer's disease. Both of these genes were described this past summer, and they have enormous implications for our understanding of this disease.

The new genes reside on chromosome 14 and chromosome 1. The chromosome 14 gene was described in June by a group in Toronto headed by St.George-Hyslop (Sherrington, 1995). Earlier workers had identified a locus on chromosome 14 that is linked to early-onset Alzheimer's disease with onset as early as age 30. This gene may account for up to 70% of familial early-onset Alzheimer's disease (which in turn is 10% of total Alzheimer's disease) (Schellenberg, 1995). Sequencing of this gene revealed a different mutation in each of five families. In each family the mutations segregated 100% with the disease, confirming the causal nature.

The mutant gene on chromosome 14 has been named S182. It encodes a protein that has all of the sequence features of a membrane protein. It has alternating hydrophobic and hydrophilic segments, indicating that it has multiple membrane spanning regions. The predicted structure is most consistent with a membrane channel, although it could be a receptor for a neurotransmitter or some other ligand. The protein is produced in brain and in most other organs. We do not know the intracellular location of this protein. All five of the mutations result in amino acid substitutions, and all five cause disease in a dominant fashion. It appears likely, therefore, that these mutations represent dominant gain-of-function mutations. They must endow the S182 protein with a new property that produces the disease.

Almost immediately after the chromosome 14 mutation was described, workers in Seattle identified a mutation in a gene on chromosome 1 that encodes a protein with high homology to the S182 protein on chromosome 14 (Levy-Lahad, 1995). The mutation was identified among individuals known as the Volga Germans. These individuals are descended from a small group of Germans who moved to Russia and settled in a region around the Volga River in the 18th century. They did not intermarry with the local population and so they retain the genetic defects of their founders. Among these is a form of early-onset Alzheimer's disease that is transmitted as an autosomal dominant trait. Through linkage studies the Seattle workers mapped this disease to chromosome 1. After the chromosome 14 gene was described, the Seattle workers searched the human genome data bank to find a sequenced gene that might be similar to the gene on chromosome 14. Indeed, they found such a gene, and they mapped it to chromosome 1, right in the region they had previously suspected that the Volga German mutation lay. They then sequenced the chromosome 1 gene from normal and affected individuals in the Volga German families. They found a point mutation that substituted an isoleucine for a highly conserved asparagine.

This sequence of events prefigures the future of human medical genetics. The human genome project is giving us an abundance of sequences along with their map locations. It will soon be commonplace to use these sequences to find new mutations as was done in this case.

The gene on chromosome 1, which is called STM2, and the gene on chromosome 14, called S182, encode highly homologous proteins that are 67% identical (Levy-Lahad, 1995). All of the

mutations in both genes occur in amino acid residues that are conserved between both proteins. It therefore appears that a dominant gain of function mutation in either STM2 or S182 can produce early-onset Alzheimer's disease.

The mutations in S182 and STM2 lead to the accumulation of the same amyloid β-protein that is found in all other forms of Alzheimer's disease (Lampe, 1994; Karlinsky, 1992). Moreover, recent unpublished studies with fibroblasts from patients with the S182 mutation indicate that these cells overproduce Aβ1-42 (R. Rosenberg, personal communication). Thus, the abnormal STM2 and S182 proteins somehow lead to the cleavage of APP to produce the 1-42 fragment which triggers the disease. But how do these mutations increase the APP processing? We don't know yet. The discovery is too new. However, a clue has emerged in the past few weeks from studies in a microscopic roundworm called *Caenorhabditis elegans*.

Scientists at Princeton have been studying the genes that lead to the formation of the vagina in hermaphroditic roundworms. Most people probably don't know that hermaphroditic roundworms have vaginas. Many mutations interfere with the formation of the vulva in these worms. One gene that is required for normal vulva formation is Sel-12. A few weeks ago this gene was cloned, and the sequence of its encoded protein was found to be 48% identical to S182, the chromosome 14 gene in Alzheimer's disease (Levitan, 1995). This is really a remarkable degree of conservation when one considers the billions of years of evolution that separate humans and roundworms. Figure 13 shows a comparison of these amino acid sequences. The identities are indicated by solid boxes.

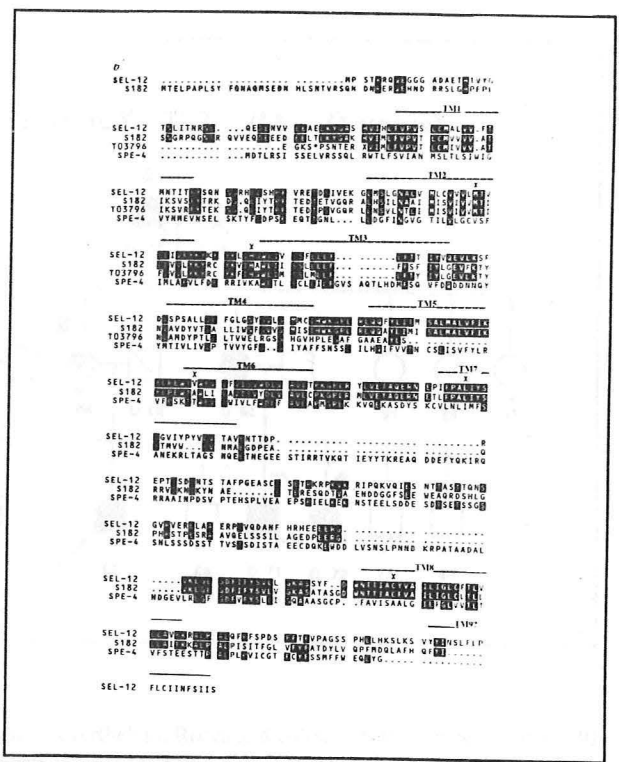

Figure 13.

We don't know precisely the function of the Sel-12 gene in *C. elegans*. Recessive loss-of-function mutations affect vulva development. However, in Alzheimer's disease the mutations are dominant gain-of-function mutations that impart a new function to this protein. It will now be necessary to reproduce these gain-of-function mutations in *C. elegans* so as to investigate what happens to the roundworm. The problem is "how will you tell when a roundworm has Alzheimer's disease?" I don't know, but it might be possible to identify an APP equivalent in *C. elegans* whose abnormal processing is accentuated by a gain-of-function mutation in Sel-12. Such a discovery would open up the biology of Alzheimer's disease to analysis by powerful genetic methods.

I would now like to turn to an even more surprising set of developments that originated far away from Princeton in the clinical department of neurology at Duke University. This work represents a nice example of clinical detective work. It deals with the association of apoE4 and late-onset Alzheimer's disease.

Allen Roses and his colleagues began by mapping a gene for late-onset Alzheimer's disease to the long arm of chromosome 19 (Perricak-Vance, 1991). This is a remarkable achievement because late-onset Alzheimer's disease involves elderly people. Alzheimer's disease is so frequent in the elderly that genetic studies are extremely difficult. Nevertheless, Roses and colleagues were able to collect 30 families with multiply affected members in which transmission appeared to be occurring through an autosomal dominant mechanism. Figure 14 shows a pedigree of one of these families.

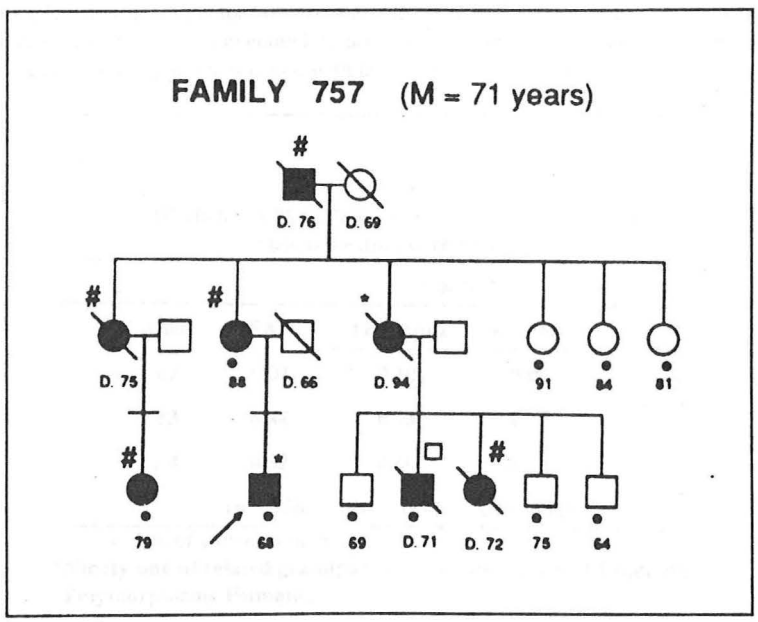

Figure 14.

While Roses was trying to figure out how he could actually identify the gene on chromosome 19, a colleague of his, Warren Strittmatter, gave him a disappointing result. Strittmatter had identified a protein in brain and cerebrospinal fluid that was able to bind the amyloid β-protein in vitro. After working for a long time he was finally able to purify this protein and he obtained its amino acid sequence. It turned out to be apolipoprotein E (apoE), a component of the blood lipoprotein transport system. ApoE circulates in blood bound to plasma lipoproteins and binds to receptors that remove these lipoproteins from the circulation. The receptors for apoE include the low density lipoprotein (LDL) receptor and the LDL Receptor Related Protein (LRP). Apo E was already known to be present in amyloid plaques of Alzheimer's disease and in many other forms of amyloidosis. Was apoE relevant to Alzheimer's?

Roses reacted to this news with great excitement. Earlier, he had noted that the gene for apoE resides on the long arm of chromosome 19 in the same region that his Alzheimer's gene was mapping. Moreover, he knew that apoE came in three varieties, designated apoE2, 3 and 4. The most common is apoE3, which accounts for 80% of alleles in most populations. ApoE4 accounts for about 14% of alleles. This was common enough to account for a large percentage of late-onset Alzheimer's disease cases. Was apoE4 associated with late-onset Alzheimer's disease?

Fortunately, there was already a simple gene-based screening method to determine whether individuals had the E4 or E3 genes. Roses immediately performed this test on affected members from his 30 families. Much to his amazement, he found a massive increase in the incidence of apoE4 among affected subjects (Figure 15). 52% of the alleles in these subjects were the E4 type, whereas these amounted to only 16% of alleles

APOE alleles of 83 patients in 30 families with familial Alzheimer disease (FAD)

		Controls	
Allele	**FAD**	**This work**	**CEPH***
ε2	**0.04**	**0.10**	**0.08**
ε3	**0.44**	**0.73**	**0.78**
ε4	**0.52**	**0.16**	**0.14**
	(n = 166)	**(n = 182)**	**(n = 2000)**

***n*, No. of chromosomes.**

***Ninety-one unrelated grandparents from the Centre d'Etude du Polymorphisme Humain.**

Figure 15.

in a control group. Since each individual has two alleles at this locus, the data indicated that more than 70% of the affected individuals in these families had at least one copy of the apoE4 gene.

These findings were so dramatic that they immediately aroused some degree of skepticism. How could this gene be making such an enormous contribution to this disease? By now even the most skeptical of the skeptics has been convinced. Roses' findings have been replicated in more than a dozen different studies from nearly every area of the world (Reviewed in Roses, 1995). Although the allele frequency in late-onset Alzheimer's may not be as high as 52% in all studies, it is always at least 40% which represents a nearly three-fold enrichment over the general population.

Figure 16 shows a recent compilation of the Duke data as it accumulated after 1991 (Saunders, 1993). Inspection reveals that the E4 gene accounts for about 41% of the alleles in late-onset Alzheimer's disease patients. It is the same irrespective of whether the patients have a family history of the disease, or whether they have no family history. The incidence of apoE4 is not increase in early-onset Alzheimer's disease, nor is it elevated in other forms of cerebral amyloidosis like Creutzfeld-Jakob disease, even though apoE is present in these lesions.

Population	No. of alleles	Age (yr) (mean)	ε4 frequency	p
Normal Controls				
CEPH grandparents	182	..	0·16	
Duke - White	98	71	0·13	NS
Duke - Black	86	71	0·17	NS
Amyloid diseases				
Creutzfeldt-Jakob				
Familial amyloidotic	20	..	0·10	NS
polyneuropathy	48	..	0·17	NS
Down's syndrome	32	..	0·09	NS
ADRC clinic				
Probable/possible AD	166	71	0·41	<0·00001*
Spouse controls	142	67	0·12	NS
AD groups				
1st affected twin	124	72	0·40	<0·00001*
CH14 or APP mutation	32	47	0·19	NS

Figure 16.

The extensive amount of data allowed Roses and colleagues to construct Kaplan-Meier plots which estimate the proportion of individuals with each genotype who will remain free of Alzheimer's disease at each age (Figure 17). The striking conclusion is that individuals with two copies of the E4 allele have a > 90% risk of developing Alzheimer's disease by age 80. Heterozygotes with the 3/4 genotype also have a high incidence of Alzheimer's disease, but it is delayed as compared with the 4/4 homozygotes. Individuals who lack E4 also develop Alzheimer's disease, but their onset is delayed by nearly 10 years when compared with the E4 patients. Notice from this graph that the presence of the third allele at this locus, namely, apoE2 seems to offer protection against Alzheimer's disease (Corder, 1994).

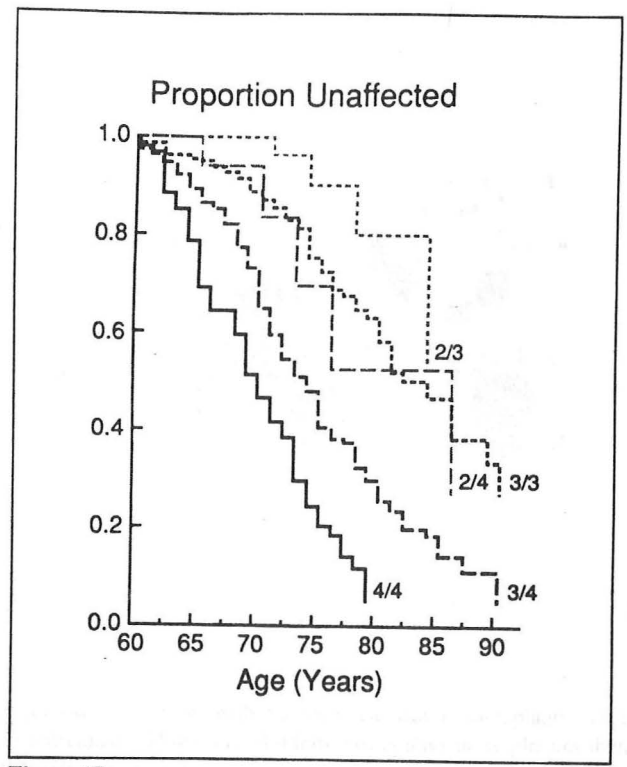

Figure 17.

Individuals who have the apoE2/4 genotype have a much later onset of disease than those who have a 3/4 genotype.

How does apoE influence Alzheimer's disease? We don't know, but one clue comes from the finding of apoE in amyloid plaques. Figure 18 shows sections of a brain of an Alzheimer's disease patient stained with an antibody against apoE (panel B) and the amyloid β-protein (panel C) (Strittmatter, 1993). The plaques stain intensely with both antibodies. Interestingly, the intensity of staining with the apoE antibody in each plaque is the same in E4/4 individuals and E3/3 individuals. However, E4/4 individuals have more plaques than E3/3 individuals (Schmechel, 1993; Hyman, 1995).

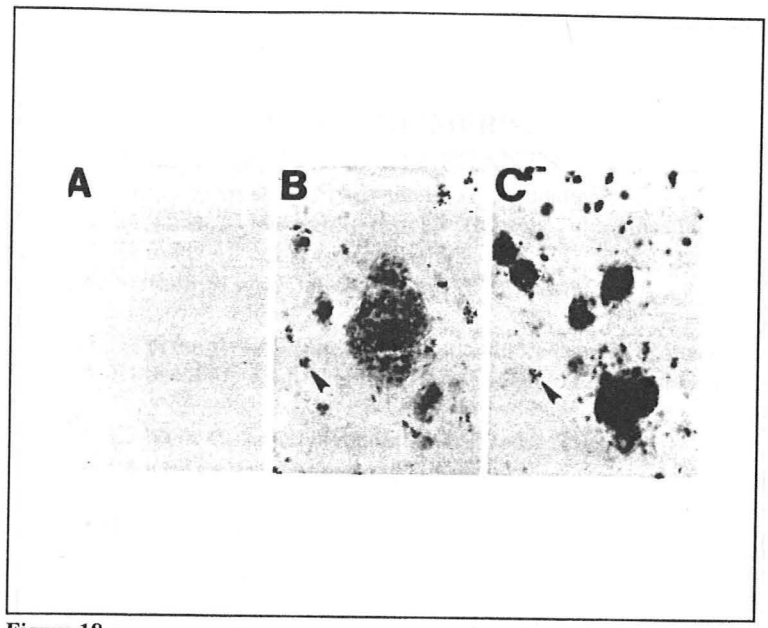

Figure 18.

The apoE4 findings set off a frenzy of experiments to try to find "the" feature of apoE4 that causes it to accelerate Alzheimer's disease. Figure 19 summarizes of all of these findings. Obviously, many of them are contradictory. ApoE4 has been reported to bind more tightly than E3 to amyloid β-protein (Strittmatter, 1993). It has also been found to bind less tightly (LaDu, 1994), equally (Naslund, 1995), or not at all (Schwarzman, 1994). Suffice it to say that we do not know the mechanism by which apoE4 and apoE3 differ in their ability to produce Alzheimer's disease. We do not even know whether E4 has a deleterious effect or whether the E4 patients suffer because they lack one or two copies of protective E3.

APO E4 AND ALZHEIMER'S:
BLIND MEN AND ELEPHANTS

1. E4 binds Aβ **more** tightly than E3 (Strittmatter)*
2. E4 binds Aβ **less** tightly than E3 (Getz)
3. E4 binds Aβ **equal to** E3 (Naslund)
4. **Neither** E4 nor E3 binds Aβ in CSF (Roses)*

4. E4 **promotes** Aβ filament formation more than E3 (Potter)
5. E4 **prevents** Aβ filament formation equal to E3 (Lansbury)

5. E3 binds tau proteins more tightly than E4 (Roses)*
6. E4 inhibits neurite outgrowth (Mahley)

*Allen Roses is co-author

Figure 19.

Figure 20 summarizes the well-established facts about apoE and Alzheimer's disease. Hopefully, in the future we will know more.

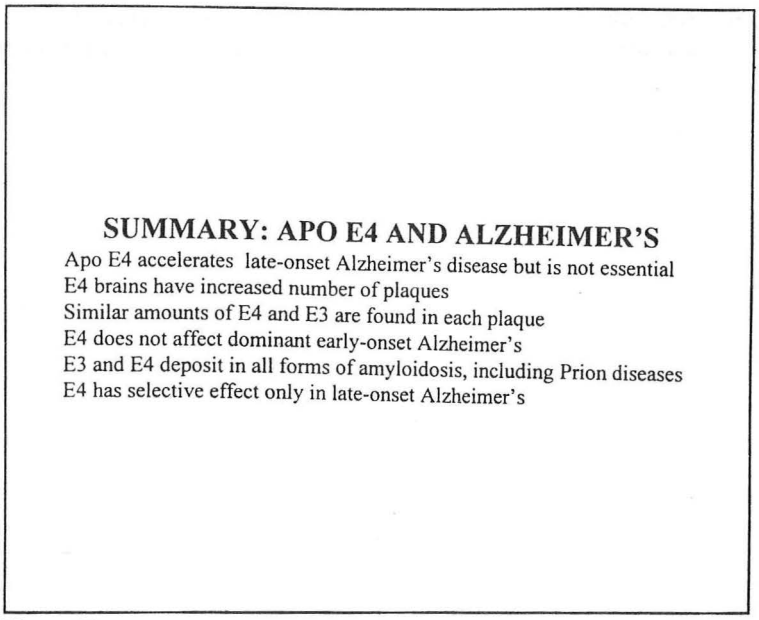

Figure 20.

The most promising arena for the elucidation of Alzheimer's disease is through the use of gene manipulated mice. Last February, a group from a biotechnology company, Athena, reported the first convincing demonstration of amyloid β-protein plaques in brains of transgenic mice (Games, 1995). They produced a mutant human APP gene with a Phe substituted for Val at position 717 which is known to increase the proportion of Aβ1-42 that is formed. They used a very strong promoter so that this protein would be produced throughout the body, including the brain. The animals produced 10-fold more precursor protein than do normal mice. When the mice were killed at 8 months of age, their brains showed classic neuritic plaques of Alzheimer's disease. Although the investigators did not report whether there was any cognitive impairment (how could they tell?), the simple finding of disease pathology will allow these mice to be used to dissect the process. It should be possible to breed these mice with mice producing apoE4, or lacking apoE altogether, to determine whether E4 makes a deleterious contribution or whether the apoE3 is helpful. Even more exciting, now that the mutations on chromosome 1 and chromosome 14 are known, it will be possible to make mutations in these genes in mice. This should lead to a much more rapid build-up of amyloid deposits.

In my opinion, there will be two major uses for the Alzheimer's mice: 1) they will help us to determine how the brain responds to amyloid β-protein deposition. We will learn whether oxidases and proteases are upregulated, and whether this upregulation contributes to the tissue damage that leads to neuronal loss. 2) We will have the first animal model in which to test therapies that break the cycle and prevent the tissue damage caused by amyloid protein deposition. This is the hope for the future.

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