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Alzheimer Lesions in the Autopsied Brains of People 30 to 50 Years of Age

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Abstract

Objective—To test the hypothesis that asymptomatic Alzheimer disease lesions may appear before 50 years of age.

Background—Alzheimer disease has an asymptomatic stage during which people are cognitively intact despite having substantial pathologic changes in the brain. While this asymptomatic stage is common in older people, how early in life it may develop has been unknown.

Methods—We microscopically examined the postmortem brains of 154 people aged 30–39 years (n = 59) and 40–50 years (n = 95) for specific Alzheimer lesions: beta-amyloid plaques, neurofibrillary tangles, and tau-positive neurites. We genotyped DNA samples for the apolipoprotein E gene (*APOE*).

Results—We found beta-amyloid lesions in 13 brains, all of them from people aged 40 to 49 with no history of dementia. These plaques were of the diffuse type only and appeared throughout the neocortex. Among these 13 brains, 5 had very subtle tau lesions in the entorhinal cortex and/or hippocampus. All individuals with beta-amyloid deposits carried 1 or 2 *APOE4* alleles. Among the individuals aged 40 to 50 with genotype *APOE3/4*, 10 (36%) had beta-amyloid deposits but 18 (64%) had none.

Conclusions—Our study demonstrates that beta-amyloid deposits in the cerebral cortex appear as early as 40 years of age in *APOE4* carriers, suggesting that these lesions may constitute a very early stage of Alzheimer disease. Future preventive and therapeutic measures for this disease may

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have to be stratified by risk factors like *APOE* genotype and target people in their 40s or even earlier.

Keywords

amyloid β ; *APOE*; preclinical Alzheimer disease; asymptomatic Alzheimer disease

INTRODUCTION

Alzheimer disease (AD), an age-associated neurodegenerative disease, is the most common cause of dementia in older people. Clinicopathologic studies indicate that by the time patients develop clinical signs of AD, the pathologic hallmarks of beta-amyloid ($A\beta$) plaques and neurofibrillary tangles in the brain are already well established. These observations have led to the widely accepted concept that the cognitive deficits of AD are preceded by a prolonged asymptomatic (Driscoll and Troncoso, 2011; Driscoll et al, 2006) or preclinical (Reiman et al, 1996; Sperling et al, 2011) period during which the pathologic changes progress slowly and silently over many years. This asymptomatic period is of great interest because it may be a critical time when disease-modifying therapies might be most effective.

The notion of asymptomatic AD emerged from autopsies, first in cross-sectional and then in longitudinal studies (Bennett et al, 2006; Crystal et al, 1988; Davis et al, 1999; Dickson et al, 1992; Miller et al, 1984; Morris and Price, 2001; Morris et al, 2001; Price et al, 2009; Sparks et al, 1993; Tomlinson et al, 1968; Troncoso et al, 1996). These studies demonstrated that neuritic beta-amyloid ($A\beta$) plaques and neurofibrillary tangles are common and frequently meet histopathologic criteria for AD in older people who have documented normal cognition before death (Bennett et al, 2006; Hulette et al, 1998; Katzman et al, 1988; Knopman et al, 2003; Morris and Price, 2001; Price and Morris, 1999; Price et al, 2009; Schmitt et al, 2000; Troncoso et al, 1998).

More recently, neuroimaging and cerebrospinal fluid (CSF) biomarker studies have also substantiated the concept of asymptomatic AD (Jack et al, 2013). In vivo imaging of brain $A\beta$ by positron emission tomography (PET) scanning (Klunk et al, 2004) has revealed $A\beta$ deposits in the brains of asymptomatic people not only in old age (Aizenstein et al, 2008; Klunk et al, 2004; Mintun et al, 2006; Resnick et al, 2010; Villemagne et al, 2008), but also in middle age (Morris et al, 2010). In asymptomatic carriers of dominant AD mutations, PET with “Pittsburgh compound B” (PiB), a ligand for $A\beta$, has demonstrated $A\beta$ deposits as early as 10 to 15 years before the expected age of symptom onset (Bateman et al, 2012).

Furthermore, an abnormally low concentration of $A\beta_{42}$ in CSF—a consistent marker of AD brain pathology (Fagan et al, 2014; Fortea et al, 2014; Gustafson et al, 2007; Li et al, 2007; Seppala et al, 2011; Shaw et al, 2009)—is seen in some normal middle-aged individuals with PiB-negative PET scans. This finding suggests that measurement of $A\beta_{42}$ in CSF may be the earliest biomarker of AD (Morris et al, 2010).

Morris et al (2010) reported that in 45- to 59-year-olds, the apolipoprotein gene (*APOE*) genotype correlates with CSF levels of $A\beta$, but not tau. The study also suggested that about

10% to 20% of individuals in this age range might have evidence of AD pathology, depending on their *APOE4* status (Corder et al, 1993; Morris et al, 2010; Reiman et al, 2009).

In the present study, we tested the hypothesis of a possible extended asymptomatic phase of AD by examining a large series of 154 postmortem brains of people who had died between the ages of 30 and 50 years. We studied the brains for AD lesions, specifically, A β plaques, neurofibrillary tangles, and tau-positive neurites. We also determined the people's *APOE* genotypes. Because we did not find A β in the 30- to 39-year-olds ($n = 59$), we focused our analysis on the 40- to 50-year-olds ($n = 95$).

A better understanding of the preclinical stages of AD will give us further insight into the pathogenesis of the disease and guide the timing of potential therapeutic interventions.

METHODS

The neuropathologic studies that we report here are part of a comprehensive program established by the Lieber Institute for Brain Development in Baltimore, Maryland.

We report a total of 154 autopsies performed between September 2012 and June 2014. These autopsies were not consecutive. Our series included a high proportion of subjects with a history of a psychiatric or behavioral abnormality. Lieber Institute staff acquired detailed information about the subjects during life.

All of the autopsies were performed in Baltimore at the Office of the Chief Medical Examiner of the State of Maryland, following protocols authorized by the Institutional Review Board of the Johns Hopkins University School of Medicine and the State of Maryland. Brain examinations and dissections were performed at the Division of Neuropathology of the Johns Hopkins University School of Medicine.

Autopsy Subjects

Table 1 shows the age, sex, and racial distribution of the 154 people whose brains we studied.

Staff at the Lieber Institute obtained a comprehensive medical history when consent for brain donation for research was granted. This information included general medical history, history of dementia and psychiatric disorders, medication history, history of alcohol consumption, use and abuse of prescribed analgesics and opiates, and use of illegal drugs. We also obtained information on history of head trauma and participation in contact sports.

The Office of the Chief Medical Examiner provided the cause and manner of death, based on a complete autopsy and toxicology examination.

Neuropathology

Brains were examined externally, weighed, and then cut into coronal slabs. Tissue blocks for histology were dissected, and other tissues were frozen. Tissue blocks for microscopic examination came from the superior frontal gyrus, middle and superior temporal gyri,

inferior parietal cortex, occipital cortex, amygdala, hippocampus and entorhinal cortex, midbrain, pons, medulla, and cerebellum. Tissues were fixed in 10% buffered formaldehyde, processed, embedded in paraffin, and cut at 10- μ m thickness. All sections were stained with hematoxylin and eosin. Selected sections were silver-stained by the Hirano method (Yamamoto and Hirano, 1986) and immunostained with antibodies against ubiquitin (Polyclonal Rabbit Anti-Ubiquitin, Dako North America, Carpinteria, California), phosphorylated monoclonal mouse anti-tau (PHF-1) (a gift of Peter Davies, PhD, Departments of Pathology and Neuroscience, Albert Einstein College of Medicine, Bronx, New York), and mouse monoclonal human against A β protein (clone: 6E10, Signet Laboratories Inc, Dedham, Massachusetts).

In brains with A β lesions, we performed single and double immunostains with antibodies for astrocytes (glial fibrillary acidic protein, DAKO North America, Carpinteria, California) and microglia (Iba1, Abcam, Cambridge, Massachusetts) to examine potential inflammatory reactions to the A β deposits.

All microscopic preparations were viewed by conventional light microscopy.

Genotyping

We genotyped all 154 brains for *APOE* by the method of Hixson and Vernier (1990).

RESULTS

A β Lesions

Among the 154 autopsied subjects, we found A β lesions in the brains of 5 women and 8 men, all of them 40 to 49 years old. In all 13 brains, the lesions were diffuse A β plaques scattered throughout the neocortex (Figure 1 and Table 2). Five of the brains showed A β lesions limited to the neocortex and not seen in the entorhinal cortex and hippocampus. In 8 brains, the A β plaques extended into the entorhinal cortex and transentorhinal cortices, and 4 of these 8 also showed plaques in the hippocampus (Table 2).

The severity of A β deposits was fairly uniform throughout the temporal, frontal, and parietal lobes, but in some brains it appeared lower, in the occipital regions. The pattern of A β deposits was also somewhat variable, ranging from very diffuse blotches of immunoreactivity to more condensed plaques (Figure 1).

We found vascular A β in 5 brains. While all 3 brains with *APOE* genotype 4/4 had vascular A β , only 2 of 10 brains with *APOE*3/4 had vascular A β (Table 2). We did not notice a correlation between vascular A β and severity of parenchymal A β deposits.

None of the 13 brains with A β deposits showed evidence of astroglial or microglial reactions.

Coexistence of A β and Tau Lesions

Among the 13 brains with A β plaques, 5 had rare and subtle tau lesions limited to the entorhinal cortex and/or hippocampus, with no involvement of the neocortex (Table 2). In 1

brain, we noted rare neurofibrillary tangles in both regions; in the other 4, the tau lesions were confined to the entorhinal cortex and consisted of punctate structures in the neuronal perikaryon and subtle neuropil threads similar to lesions in stages 1a and 1b of Braak et al (2011) (Figure 2). We saw no tau lesions in the neocortex. The remaining 8 brains bearing A β plaques were free of tau abnormalities in the cerebral cortex.

APOE4 Genotypes

As shown in Table 2, all 13 individuals whose brain had A β deposits were 40 to 49 years of age and all carried 1 or 2 *APOE4* alleles.

For our total series of 154 brains, Table 3 shows the distribution of *APOE* alleles by age group and race. We found no significant differences in the *APOE* allele frequency between the subjects 30 to 39 years old and those 40 to 50 years old, or between whites and African Americans.

Table 4 shows the *APOE* genotype distribution in the 95 subjects aged 40 to 50, with and without A β . All 3 brains with *APOE4/4* had A β deposits. Of the brains with *APOE3/4*, 10 (36%) had A β deposits, but 18 (64%) did not. The brains with other genotypes, including *APOE 2/4*, were free of A β .

Table 5 lists the cause and manner of death for each of the 13 subjects with A β deposits. As outlined in Table 6, we did not observe any significant differences in the manner of death between the subjects with and those without A β deposits.

Table 7 compares the medical histories for the 40- to 50-year olds by their A β status. The histories were similar except for more heart disease in the A β -positive group.

DISCUSSION

Our major finding in this study was A β deposits in 13 of 95 (13.7%) autopsied brains from non-demented individuals between 40 and 50 years of age. The frequency of A β lesions in this age range was 13.3% in men, 14.3% in women, 15.5% in whites, and 10.4 % in African Americans. We did not find A β deposits in any individual younger than 40.

This last observation is consistent with a study by Braak et al (2011) reporting that A β deposits started to appear at approximately age 40 and affected 4% of individuals between 40 and 50. Previous studies from our laboratory have also shown that A β lesions started to appear in the early 40s, and their frequency was 7.8% in 40- to 54-year-olds, without differences between the sexes or races (Sandberg et al, 2001).

Therefore, it appears that the beginning of the fifth decade is the inflection point for development of A β deposits and senile plaques, which may be advanced by genetic factors such as Down syndrome (Burger and Vogel, 1973; Cork, 1990; Mann and Esiri, 1989) or amyloid precursor protein and presenilin mutations (Rogaeva, 2002).

The A β lesions that we saw in this study were diffuse plaques, some perineuronal, and not associated with obvious neuritic or inflammatory components. These plaques were similar to

ones that we had reported in the preclinical stages of AD, associated with endosomal and lysosomal abnormalities (Troncoso et al, 1998). We found the preclinical plaques throughout the neocortex, although less frequently in occipital regions.

Because in the present study we found these lesions only in the subjects aged 40 or older, the lesions likely represent the very early stages of AD pathology. Our belief is reinforced by finding that each of the affected individuals had at least 1 *APOE4* allele.

Notably, only 5 of the 13 brains with A β deposits had any tau pathology in the cerebral cortex. The tau lesions were confined to the mesial temporal lobe. None were in the neocortex, where A β deposition was most severe. This strongly suggests that A β deposition is the earliest event in AD, preceding the development of local tau lesions and neurofibrillary tangles.

Our observations support the amyloid hypothesis of the pathogenesis of AD, which proposes that abnormal A β processing, deposition, and clearing are the primordial events in AD, coming before inflammatory changes, tau lesions, and neuronal degeneration (Hardy, 2009; Hardy and Higgins, 1992; Selkoe, 1991).

Few studies have been done of AD biomarkers in normal young people. We compared our findings to those of Morris et al's (2010) clinical biomarkers study in cognitively normal aging, and we found both differences and similarities. We detected A β deposits in 13.7% of our subjects 40 to 50 years of age, but Morris et al did not find any A β deposits on PiB PET scans of subjects aged 45 to 49. This discrepancy suggests that PiB PET scans may not be sensitive enough to detect the very early stages of A β deposition.

Our results concur with the finding by Morris et al that 18.2% of their subjects had low CSF A β 42 concentrations. Both studies support the notion that this biomarker is sensitive to very early A β deposition. Studies of CSF biomarkers in autosomal-dominant AD have associated reduced CSF A β 42 with A β plaques as well as with elevated CSF tau in asymptomatic mutation carriers; both markers can be found 10 to 20 years before the estimated age of onset (Fagan et al, 2014).

The *APOE4* allele is the major known risk factor for the development of late-onset sporadic AD (Corder et al, 1993). This effect may be mediated by the increased A β deposition in the brain and by changes in synaptic repair and plasticity (Verghese et al, 2011). Furthermore, individuals carrying the *APOE4* allele deposit A β in the brain in a dose-dependent manner during the preclinical stages of the disease (Morris et al, 2010; Reiman et al, 2009).

In our sample, all subjects with A β deposits in the brain carried 1 or 2 *APOE4* alleles, reaffirming the importance of *APOE4* for A β deposition (Corder et al, 1993). All 3 of our subjects who had the E4/E4 genotype had A β deposits. Although our number of *APOE4*-homozygous subjects was small, the finding that 100% of them had A β deposits versus only 36% of subjects with *APOE3/4* is congruent with the concept of the gene dose effect of *APOE* (Morris et al, 2010; Reiman et al, 2009).

Based on our cross-sectional observation, we cannot predict whether these individuals, had they lived longer, would have developed cognitive decline or dementia. However, considering the epidemiologic data on the *APOE4* carriers, some probably would have. Importantly, many of the subjects bearing a single *APOE4* allele did not show A β deposits, suggesting that other genetic (Guerreiro et al, 2012, 2013) and environmental (Wirth et al, 2014) factors can modulate the risk effect of *APOE* for A β deposition and development of AD.

We also note that the frequency of the E4 allele in our African American subjects aged 40 to 50 was 19.6%, consistent with the 19.5% frequency reported in a previous study (Riudavets et al, 2006).

A major limitation of our study was the nature of the sample. People coming to autopsy in a medical examiner's office do not necessarily represent the population at large, and, in particular, may include a disproportionate number of men and women with a history of substance abuse and/or mental health disorders. This limitation, however, is balanced by the opportunity to examine a large number of brains in a short period of time. Furthermore, the composition of our sample is valuable as it includes a large proportion of African Americans, who have not been well represented in autopsy studies of AD.

In conclusion, our study demonstrates that *APOE4* carriers as young as 40 years of age may have asymptomatic or preclinical AD characterized by A β deposits in the cerebral cortex. Our finding highlights the need to target future AD preventive and therapeutic measures at young people, perhaps triaged on the basis of genetic risk, and the need to develop sensitive biomarkers of AD that can be applied to people in their 40s or even younger.

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Glossary

AD	Alzheimer disease
Aβ	beta amyloid
APOE	apolipoprotein gene
CSF	cerebrospinal fluid
PET	positron emission tomography
PiB	Pittsburgh compound B

REFERENCES

Aizenstein HJ, Nebes RD, Saxton JA, et al. Frequent amyloid deposition without significant cognitive impairment among the elderly. *Arch Neurol*. 2008; 65:1509–1517. [PubMed: 19001171]

- Bateman RJ, Xiong C, Benzinger TL, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med*. 2012; 367:795–804. [PubMed: 22784036]
- Bennett DA, Schneider JA, Arvanitakis Z, et al. Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology*. 2006; 66:1837–1844. [PubMed: 16801647]
- Braak H, Thal DR, Ghebremedhin E, et al. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *J Neuropathol Exp Neurol*. 2011; 70:960–969. [PubMed: 22002422]
- Burger PC, Vogel FS. The development of the pathologic changes of Alzheimer's disease and senile dementia in patients with Down's syndrome. *Am J Pathol*. 1973; 73:457–476. [PubMed: 4271339]
- Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*. 1993; 261:921–923. [PubMed: 8346443]
- Cork LC. Neuropathology of Down syndrome and Alzheimer disease. *Am J Med Genet Suppl*. 1990; 7:282–286. [PubMed: 2149963]
- Crystal H, Dickson D, Fuld P, et al. Clinico-pathologic studies in dementia: nondemented subjects with pathologically confirmed Alzheimer's disease. *Neurology*. 1988; 38:1682–1687. [PubMed: 3185902]
- Davis DG, Schmitt FA, Wekstein DR, et al. Alzheimer neuropathologic alterations in aged cognitively normal subjects. *J Neuropathol Exp Neurol*. 1999; 58:376–388. [PubMed: 10218633]
- Dickson DW, Crystal HA, Mattiace LA, et al. Identification of normal and pathological aging in prospectively studied nondemented elderly humans. *Neurobiol Aging*. 1992; 13:179–189. [PubMed: 1311804]
- Driscoll I, Resnick SM, Troncoso JC, et al. Impact of Alzheimer's pathology on cognitive trajectories in nondemented elderly. *Ann Neurol*. 2006; 60:688–695. [PubMed: 17192929]
- Driscoll I, Troncoso J. Asymptomatic Alzheimer's disease: a prodrome or a state of resilience? *Curr Alzheimer Res*. 2011; 8:330–335. [PubMed: 21222594]
- Fagan AM, Xiong C, Jasielec MS, et al. Longitudinal change in CSF biomarkers in autosomal-dominant Alzheimer's disease. *Sci Transl Med*. 2014; 6:226–230.
- Fortea J, Vilaplana E, Alcolea D, et al. Cerebrospinal fluid beta-amyloid and phospho-tau biomarker interactions affecting brain structure in preclinical Alzheimer disease. *Ann Neurol*. 2014; 76:223–230. [PubMed: 24852682]
- Guerreiro R, Bras J, Hardy J. SnapShot: genetics of Alzheimer's disease. *Cell*. 2013; 155:968–968.e961. [PubMed: 24209629]
- Guerreiro RJ, Gustafson DR, Hardy J. The genetic architecture of Alzheimer's disease: beyond APP, PSENs and APOE. *Neurobiol Aging*. 2012; 33:437–456. [PubMed: 20594621]
- Gustafson DR, Skoog I, Rosengren L, et al. Cerebrospinal fluid beta-amyloid 1-42 concentration may predict cognitive decline in older women. *J Neurol Neurosurg Psychiatry*. 2007; 78:461–464. [PubMed: 17098843]
- Hardy J. The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. *J Neurochem*. 2009; 110:1129–1134. [PubMed: 19457065]
- Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science*. 1992; 256:184–185. [PubMed: 1566067]
- Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res*. 1990; 31:545–548. [PubMed: 2341813]
- Hulette CM, Welsh-Bohmer KA, Murray MG, et al. Neuropathological and neuropsychological changes in “normal” aging: evidence for preclinical Alzheimer disease in cognitively normal individuals. *J Neuropathol Exp Neurol*. 1998; 57:1168–1174. [PubMed: 9862640]
- Jack CR Jr, Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol*. 2013; 12:207–216. [PubMed: 23332364]
- Katzman R, Terry R, DeTeresa R, et al. Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. *Ann Neurol*. 1988; 23:138–144. [PubMed: 2897823]

- Klunk WE, Engler H, Nordberg A, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann Neurol*. 2004; 55:306–319. [PubMed: 14991808]
- Knopman DS, Parisi JE, Salviati A, et al. Neuropathology of cognitively normal elderly. *J Neuropathol Exp Neurol*. 2003; 62:1087–1095. [PubMed: 14656067]
- Li G, Sokal I, Quinn JF, et al. CSF tau/Abeta42 ratio for increased risk of mild cognitive impairment: a follow-up study. *Neurology*. 2007; 69:631–639. [PubMed: 17698783]
- Mann DM, Esiri MM. The pattern of acquisition of plaques and tangles in the brains of patients under 50 years of age with Down's syndrome. *J Neurol Sci*. 1989; 89:169–179. [PubMed: 2522541]
- Miller FD, Hicks SP, D'Amato CJ, et al. A descriptive study of neuritic plaques and neurofibrillary tangles in an autopsy population. *Am J Epidemiol*. 1984; 120:331–341. [PubMed: 6332530]
- Mintun MA, Larossa GN, Sheline YI, et al. [11C]PIB in a nondemented population: potential antecedent marker of Alzheimer disease. *Neurology*. 2006; 67:446–452. [PubMed: 16894106]
- Morris JC, Price JL. Pathologic correlates of nondemented aging, mild cognitive impairment, and early-stage Alzheimer's disease. *J Mol Neurosci*. 2001; 17:101–118. [PubMed: 11816784]
- Morris JC, Roe CM, Xiong C, et al. APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. *Ann Neurol*. 2010; 67:122–131. [PubMed: 20186853]
- Morris JC, Storandt M, Miller JP, et al. Mild cognitive impairment represents early-stage Alzheimer disease. *Arch Neurol*. 2001; 58:397–405. [PubMed: 11255443]
- Price JL, McKeel DW Jr, Buckles VD, et al. Neuropathology of nondemented aging: presumptive evidence for preclinical Alzheimer disease. *Neurobiol Aging*. 2009; 30:1026–1036. [PubMed: 19376612]
- Price JL, Morris JC. Tangles and plaques in nondemented aging and “preclinical” Alzheimer's disease. *Ann Neurol*. 1999; 45:358–368. [PubMed: 10072051]
- Reiman EM, Caselli RJ, Yun LS, et al. Preclinical evidence of Alzheimer's disease in persons homozygous for the epsilon 4 allele for apolipoprotein E. *N Engl J Med*. 1996; 334:752–758. [PubMed: 8592548]
- Reiman EM, Chen K, Liu X, et al. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2009; 106:6820–6825. [PubMed: 19346482]
- Resnick SM, Sojkova J, Zhou Y, et al. Longitudinal cognitive decline is associated with fibrillar amyloid-beta measured by [11C]PiB. *Neurology*. 2010; 74:807–815. [PubMed: 20147655]
- Riudavets MA, Rubio A, Cox C, et al. The prevalence of Alzheimer neuropathologic lesions is similar in blacks and whites. *J Neuropathol Exp Neurol*. 2006; 65:1143–1148. [PubMed: 17146288]
- Rogaeva E. The solved and unsolved mysteries of the genetics of early-onset Alzheimer's disease. *Neuromolecular Med*. 2002; 2:1–10. [PubMed: 12230301]
- Sandberg G, Stewart W, Smialek J, et al. The prevalence of the neuropathological lesions of Alzheimer's disease is independent of race and gender. *Neurobiol Aging*. 2001; 22:169–175. [PubMed: 11182466]
- Schmitt FA, Davis DG, Wekstein DR, et al. “Preclinical” AD revisited: neuropathology of cognitively normal older adults. *Neurology*. 2000; 55:370–376. [PubMed: 10932270]
- Selkoe DJ. The molecular pathology of Alzheimer's disease. *Neuron*. 1991; 6:487–498. [PubMed: 1673054]
- Seppala TT, Koivisto AM, Hartikainen P, et al. Longitudinal changes of CSF biomarkers in Alzheimer's disease. *J Alzheimers Dis*. 2011; 25:583–594. [PubMed: 21460434]
- Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol*. 2009; 65:403–413. [PubMed: 19296504]
- Sparks DL, Liu H, Scheff SW, et al. Temporal sequence of plaque formation in the cerebral cortex of non-demented individuals. *J Neuropathol Exp Neurol*. 1993; 52:135–142. [PubMed: 8440995]
- Sperling RA, Jack CR Jr, Black SE, et al. Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials: recommendations from the Alzheimer's Association Research Roundtable Workgroup. *Alzheimers Dement*. 2011; 7:367–385. [PubMed: 21784348]

- Tomlinson BE, Blessed G, Roth M. Observations on the brains of non-demented old people. *J Neurol Sci.* 1968; 7:331–356. [PubMed: 5707082]
- Troncoso JC, Cataldo AM, Nixon RA, et al. Neuropathology of preclinical and clinical late-onset Alzheimer's disease. *Ann Neurol.* 1998; 43:673–676. [PubMed: 9585365]
- Troncoso JC, Martin LJ, Dal Forno G, et al. Neuropathology in controls and demented subjects from the Baltimore Longitudinal Study of Aging. *Neurobiol Aging.* 1996; 17:365–371. [PubMed: 8725897]
- Verghese PB, Castellano JM, Holtzman DM. Apolipoprotein E in Alzheimer's disease and other neurological disorders. *Lancet Neurol.* 2011; 10:241–252. [PubMed: 21349439]
- Villemagne VL, Pike KE, Darby D, et al. Abeta deposits in older non-demented individuals with cognitive decline are indicative of preclinical Alzheimer's disease. *Neuropsychologia.* 2008; 46:1688–1697. [PubMed: 18343463]
- Wirth M, Villeneuve S, La Joie R, et al. Gene-environment interactions: lifetime cognitive activity, APOE genotype, and beta-amyloid burden. *J Neurosci.* 2014; 34:8612–8617. [PubMed: 24948815]
- Yamamoto T, Hirano A. A comparative study of modified Bielschowsky, Bodian and thioflavin S stains on Alzheimer's neurofibrillary tangles. *Neuropathol Appl Neurobiol.* 1986; 12:3–9. [PubMed: 2422580]

IN MEMORIAM

This work is dedicated to Dr Oscar S.M. Marin, my mentor, friend, and fellow countryman.

I went to medical school in Santiago, Chile, at the Universidad Católica, where Oscar Marin had been Professor of Neurology from 1961-1965. I arrived in 1967. During medical school, although I was supposed to become a surgeon, I fell in love with neurology and devoted as much time as possible to getting experience in neurology and neuroscience.

Only years later did I recognize how much Dr Marin had shaped both the clinical and academic environment in which I had developed my interest. He had either created or heavily influenced the hospital neurology services in which I learned, and he had taught my professors. Dr Jaime Court, my professor of neurology and mentor, had been a student of Dr Marin and coauthored a 1965 paper with him on necrotizing arteries.

After I graduated in 1973, I wished to enter a neurology training program in Santiago. But 1973 was also the year of the military coup in Chile. Things got quite difficult, and my wife, Gloria, and I decided to leave the country.

I wrote to many institutions outside Chile before finding help from a friend of mine, Dr Fernando Miranda, who was doing a neurology residency at the University of Maryland in Baltimore [see Dr Miranda's article in this issue of the journal]. Dr Miranda helped me obtain an internship in internal medicine at the South Baltimore General (now Harborview) Hospital. This internship was a prerequisite for applying for a neurology training program.

We arrived in Baltimore in the last week of June 1974—Gloria, who was pregnant with our future son Felipe, and I. On July 1, I began working at South Baltimore General Hospital.

After settling for a few weeks, I decide to visit Dr Marin, who was then on the faculty at the Johns Hopkins University School of Medicine in Baltimore. It was probably late July when I drove to the then Baltimore City Hospitals to meet Dr Marin. I brought a letter of reference from Dr Juan Allamand, a renowned Chilean surgeon who knew him.

I explained my situation to Dr Marin and asked him for advice about seeking training in neurology. He was very warm and receptive. He gave me his perspective on the current neurology training landscape and recommended 3 or 4 good East Coast neurology programs where I would be competitive. One of his recommendations was Hahnemann University Hospital in Philadelphia, where I eventually did my neurology residency under the mentorship of Dr Elliott Mancall.

In addition to giving professional advice, Dr Marin invited Gloria and me to visit him at his home. That was when we met the women of the Marin family, Señora Clara, Clarita, and Andrea (Oscar Jr was away). We saw each other socially several times. Then the Marins asked us if we would house-sit for them in December and January, while they stayed at their apartment in St Denis, Paris. To make a long story short, we were living at the Marins' house when Felipe was born in early January of 1975.

That July, we moved to Philadelphia for my neurology residency. There I became very interested in neuropathology while working for 6 months with Dr David Dunn at the Medical College of Pennsylvania. In 1978, at the end of my clinical training, Dr Marin helped me get a neuropathology fellowship at Johns Hopkins. He was one of my sponsors, along with Drs Donald Price and Guy McKhann. Shortly after I began my neuropathology training, Dr Marin asked me to start doing brain cuttings and neuropathology conferences with him at Baltimore City Hospitals. During my second year, I also began attending as a clinical neurologist at Baltimore City Hospitals.

After Dr Marin left for Portland, Oregon, in 1979, I visited him several times. I have stayed on at Johns Hopkins.

In retrospect, I appreciate how much Dr Marin influenced my career even before I met him, because he was largely responsible for the thriving neurology environment that so excited me in medical school in Chile. Then, as I moved to the US, he gave me good advice and strongly supported my professional development. Dr Marin was a thoughtful mentor who guided my career wisely and at the same time was a wonderful friend.

— Juan C. Troncoso, MD

FIGURE 1.

Photomicrographs of neocortex from one of the autopsied subjects. The sections are immunostained with 6E10 antibody, revealing beta amyloid ($A\beta$) deposits and plaques. Panel A: Rare plaques. Panel B: Frequent plaques. Panel C: High magnification of a diffuse plaque shrouding several neurons. Panel D: $A\beta$ deposit in the wall of a blood vessel.

FIGURE 2.

Photomicrographs of hippocampus from one of the autopsied subjects. The sections are immunostained with phospho-tau antibody. Panel A: Pyramidal neuron with immunoreactive organelles in the perikaryon and proximal dendrites. Panel B: Neuropil threads in the entorhinal cortex.

TABLE 1

Age, Sex, and Race of 154 Autopsied Subjects

	Age (Years)		Total (n = 154)
	30-39 (n = 59)	40-50 (n = 95)	
Sex			
Men	36	60	96
Women	23	35	58
Race			
White	41	66	107
African American	14	28	42
Other	4	1	5

Demographic Information, Neuropathologic Findings, and Apolipoprotein E (*APOE*) Genotypes of the 13 Autopsied Subjects Aged 40 to 50 Years Who Had Beta Amyloid (A β) Lesions

TABLE 2

			Location and Severity of Lesions												APOE Genotype
Subject	Age, Sex	Race	Aβ										* Tau		
			Hippocampus	Transentorhinal Cortex	Entorhinal Cortex	Inferior Temporal Gyrus	Frontal Cortex	Superior & Middle Temporal Gyri	Inferior Parietal Lobule	Occipital Cortex	Blood Vessels	Mesial Temporal Lobe	Neocortex		
1	47, W	AA	–	+	+	+	+	+	+	+	+	++	–	–	E4/E4
2	43, M	W	+	na	++	na	++	++	+++	+++	+	+	–	–	E4/E4
3	49, M	W	+	+	+	+++	+++	+++	+++	+++	+++	+	–	–	E4/E4
4	46, W	W	+	+	+	++	+++	+++	+++	+++	+++	–	–	–	E3/E4
5	43, M	W	na	na	na	na	++	++	+++	+++	++	–	–	–	E3/E4
6	47, M	W	–	–	–	+	+	+	+	+	+	–	–	–	E3/E4
7	44, M	AA	–	+++	++	+++	++	++	+++	+++	++	–	+	–	E3/E4
8	40, M	W	–	–	–	++	+	+	+	+	+	–	–	–	E3/E4
9	45, W	W	–	+++	+++	na	++	++	+	+	+	+	+	–	E3/E4
10	45, W	W	–	++	–	++	+	+	+++	+++	+	–	–	–	E3/E4
11	48, W	W	–	+	+	+++	++	++	++	++	++	+	+	–	E3/E4
12	44, M	AA	+	+	+	+++	++	++	+++	+++	+++	–	+	–	E3/E4
13	47, M	W	–	–	–	+	+	+	–	–	+	–	+	–	E3/E4

W = woman. M = man. AA = African American. W = white. A β = beta amyloid protein. na = not available. Mesial Temporal Lobe = hippocampus and/or entorhinal cortex.

* Number of lesions: – = none. + = rare. ++ = moderate. +++ = frequent.

TABLE 3Apolipoprotein E (*APOE*) Gene Alleles in Autopsied Subjects Aged 30-39 versus 40-50 Years

Race	# of Alleles	APOE Allele		
		E2	E3	E4
59 subjects aged 30-39 APOE alleles: 59 × 2 = 118				
White	82	9 (10.9%)	59 (71.9%)	14 (17%)
African American	28	3 (10.7%)	20 (71.4%)	5 (17.9%)
Other	8	0	8 (100%)	0
Total	118	12 (10.17%)	87 (73.7%)	19 (16.1%)
95 subjects aged 40-50 APOE alleles: 95 × 2 = 190				
White	132	13 (9.8%)	93 (70.4%)	26 (19.7%)
African American	56	7 (12.5%)	38 (67.8%)	11 (19.6%)
Other	2	0	2 (100%)	0
Total	190	20 (10.5%)	133 (70%)	37 (19.5%)

TABLE 4

Apoprotein E (*APOE*) Genotypes in 95 Autopsied Subjects Aged 40-50 Years, by Beta Amyloid ($A\beta$) Status

<i>APOE</i> Genotypes	n	$A\beta$ Status	
		Positive	Negative
2/2	2	0 (0%)	2 (100%)
2/3	13	0 (0%)	13 (100%)
2/4	3	0 (0%)	3 (100%)
3/3	46	0 (0%)	46 (100%)
3/4	28	10 (36%)	18 (64%)
4/4	3	3 (100%)	0 (0%)
Total	95	13 (13.5%)	83 (86.4%)

TABLE 5Cause and Manner of Death in 13 Autopsied Subjects with A β Deposits

Subject #	Cause of Death	Manner of Death
1	Bupropion intoxication	Suicide
2	Narcotic intoxication	Undetermined
3	Multiple injuries	Accident
4	Arteriosclerotic cardiovascular disease and narcotic intoxication	Undetermined
5	Fentanyl and chlordiazepoxide intoxication	Undetermined
6	Drowning, alcohol and mixed drug intoxication	Undetermined
7	Drowning	Accident
8	Alcohol and narcotic intoxication	Undetermined
9	Cardiac arrhythmia	Natural
10	Arteriosclerotic cardiovascular disease	Natural
11	Arteriosclerotic cardiovascular disease	Natural
12	Intraoral gunshot wound	Suicide
13	Megacolon	Natural

TABLE 6Manner of Death and Beta Amyloid (A β) Status of 95 Autopsied Subjects Aged 40-50 Years

Manner of Death	A β Status	
	Negative (n = 82)	Positive (n = 13)
Natural	40 (48.8%)	4 (30.8%)
Accident	13 (15.9%)	2 (15.3%)
Suicide	9 (10.9%)	2 (15.3%)
Undetermined	20 (24.4%)	5 (38.4%)

TABLE 7

Medical History of 95 Autopsied Subjects Aged 40-50 Years, by Beta Amyloid (A β) Status

History	A β Status	
	Negative (n = 82)	Positive (n = 13)
Schizophrenia	5%	8%
Affective disorder	42%	31%
Alcohol abuse	23%	23%
Drug abuse	31%	23%
Use of narcotic analgesics	2%	8%
Traumatic brain injury	1%	0%
Post-traumatic stress disorder	6%	0%
Suicide attempts	4%	8%
Heart disease	4%	23%

Some subjects had > 1 psychiatric disorder, traumatic brain injury, or type of cardiovascular disease.