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The Framingham Brain Donation Program: Neuropathology Along the Cognitive Continuum

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Abstract

The Framingham Heart Study has enrolled 3 generations of participants, the Original cohort (Gen 1) enrolled in 1948, the Offspring cohort (Gen 2) enrolled in 1971 and the Third Generation enrolled in 2002. Participants have been undergoing prospective surveillance for incident stroke and dementia and embedded within this cohort is the voluntary Framingham Brain Donation Program that was begun in 1997. Participants who register to become brain donors have had one or more brain MR and cognitive test batteries administered. In addition, they undergo neurological evaluation as indicated, record review and post-mortem next-of-kin interview to determine the presence, type and extent of antemortem, clinical neurological diagnoses and to assign a retrospective Clinical Dementia Rating (CDR) Scale score. Between 1997 and 2009 there were 1806 deaths, 186 of which were among registered brain donors and of these 139 brains could be examined. 58% were deemed cognitively normal at death. We present results for 3 projects; the first was to examine the sensitivity and specificity of our clinical diagnosis against the gold standard of pathological AD in 59 persons who underwent detailed cognitive assessment in the two years prior to death; we observed a 77.3% sensitivity (2 persons with AD were diagnosed clinically as Lewy body dementia) and a 91.9% specificity. The second examined the correlation of regional Alzheimer-type pathology to cognitive status at death among 34 persons who were over the age of 75 and without any significant vascular or alternative neurodegenerative pathology and found that neurofibrillary tangle counts distinguished between persons who were controls, had mild cognitive impairment, mild or moderate dementia; tangles in dorsolateral frontal cortex best distinguished MCI and controls. The third project examined the extent and severity of vascular pathology, again in a larger sample of varying cognitive abilities and in a subsample of persons with either amnestic or non-amnestic MCI. We observed that an aggregate ischemic injury score was significantly higher in persons with a CDR score of 0.5 than in normal controls.

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Keywords

Brain; autopsy; epidemiology; alzheimer's disease; brain ischemia

Introduction

The Framingham Heart Study (FHS) began in 1948 as a study of risk factors for incident heart disease, enrolling twothirds of all participants residing at that time in the town of Framingham, Massachusetts. Biennial examination of these original participants (Gen 1) has generated an extensive database of cardiovascular and cerebrovascular risk factors that spans more than 6 decades. In 1971, a second generation, the biological children of the Original cohort and their spouses (Gen 2), were recruited and have completed eight regular health examinations. A third generation, the grandchildren of the Original cohort (Gen 3), were invited to join the study, and began participation in 2002. Gen 3 participants were also required to have at least one parent enrolled in the Gen 2 sample and priority was given to persons from large families. Details of sample selection and enrollment have been published previously [1-3].

The systematic study of dementia in FHS began in 1974-76 with a baseline neuropsychological evaluation of the Gen 1 cohort, which established a dementia-free inception cohort that has since been followed for incident disease. The introduction of the Mini-Mental State Examination (MMSE) in 1981 allowed for on-going screening of cognitive status at each biennial examination. Similarly, since 1991, the MMSE has been added to consecutive Gen 2 examinations to document mental status and to monitor changes. Details of the dementia tracking and surveillance mechanisms at FHS have been published previously. In 1997 the FHS began a postmortem brain tissue donation program in collaboration with the Boston University Alzheimer's Disease Center's Neuropathology Core. Participants are informed of this program through periodic newsletters, and those who call to express interest are sent additional brochures and informational materials. Such material is also shared when participants come in for neuropsychological (NP), brain MRI or neurological testing. Participants who wish to sign up are also urged to inform their families of their wishes. We collect and periodically update contact information including details regarding their next-of-kin. We also provide a single toll-free telephone number that the family can call to report a death. Enrolled participants are invited to undergo cognitive testing and brain MRI, and when indicated neurological assessment, once every 1-2 years. Out-of-state participants and those unable to come into the clinic are tested in their places of residence.

In June 2009, we had 35 Gen 1 participants, all over 90 years of age, and 398 Gen 2 subjects (32% of whom were over 75 years old) enrolled as potential brain donors. This included 16% and 11% of surviving members of the Gen 1 and Gen 2 cohorts, respectively.

One of the important characteristics of the FHS' brain donation program is that it is embedded within an ongoing cohort study; hence about half of the autopsy cases come from individuals who were clinically determined to be cognitively intact at the time of death. Further, all persons have extensive lifestyle and risk factor data gathered over their adult lifespan and most also have antemortem imaging, neurological and neuropsychological (NP) assessment data. As of June 2009 we had obtained 15 antemortem scans and 59 NP assessments within 2 years of death. Further, each one of the Gen 1 and Gen 2 participants enrolled has had at least 1 brain MRI and 1 NP examination. Among Gen 1, 22 (63%) have had 2 or more brain MRI scans and 34 (97%) have had 2 or more NP examinations. Among

Gen 2 subjects, 293 (75%) have had 2 or more MRI scans and 343 (88%) have had 2 or more NP examinations.

Over the 12 years since the Framingham Brain Donation Program began (January 1997 to June 2009), we have had total of 1804 deaths in Gen 1 and Gen 2 participants; of these 186 deaths (10%) were among registered brain donors. Of the latter, we have received 139 brains (74%), and detailed neuropathology reports are available for all cases. 58% of the brains analyzed were deemed pathologically normal.

In this paper we outline the neuropathological protocols we use and describe 3 projects that utilized these FHS brain bank data, they were undertaken at different times over this period and have differing sample frames. The first project was to validate our clinical diagnosis and was undertaken on 59 persons (of 92 who died between 1997 and 2002) who underwent detailed cognitive assessment in the two years prior to death. The second aimed to examine the relative neuropathological contributions of different Alzheimer type pathological changes to cognitive function in persons over age 75 years, both in a larger sample of persons with varying degrees of cognitive impairment and specifically among persons with antemortem mild cognitive impairment (MCI). For this project data from 34 persons (out of 42 brain donors over age 75 who died between 1997 and 2003) with no confounding pathology such as a second neurodegenerative condition or significant vascular disease was evaluated. The third project was to examine the extent and severity of vascular pathology, again in a larger sample of varying cognitive abilities and in a subsample of persons with MCI.

Methods

Details of the establishment and ongoing surveillance of the FHS' dementia-free cohort have been described previously [4-7]. Participants found to be cognitively impaired are referred to the FHS' neurological group (RA, SS, SA, PAW) for annual neurological and NP examinations. If either the neurological or NP examination indicates the possibility of dementia, the case records are brought to a diagnostic review meeting. At this consensus conference, the diagnostic protocol requires review of all available medical information from 5 key sources: 1) FHS' health examinations, 2) FHS neurological examination, 3) NP assessments, 4) outside medical records and nursing home records and 5) for those whose brains come to autopsy, a post-mortem family interview. Post-mortem family interviews are generally conducted with the closest next of kin and include inquiries about changes in cognitive and functional status, and the time line associated with these changes. Further, questions from the Blessed Dementia Rating Scale [8], the Hachinski ischemic score [9] and the Retrospective Clinical Dementia Rating Scale [10] are embedded in the Family Interview questionnaire. Presence or absence of dementia and the type of dementia are determined based on clinical information alone, and the clinical diagnosis is only shared with the neuropathologist at a clinico-pathological conference prior to which all pathological data and diagnoses have been independently recorded.

The severity of the cognitive impairment at death is categorized as mild cognitive impairment (MCI, usually corresponding to a CDR of 0.5) or dementia of varying severity (mild, moderate, severe corresponding roughly to CDR scores of 1, 2 and ≥3).

Diagnostic Criteria for Dementia, Alzheimer's Disease and MCI

All individuals identified as having dementia satisfy DSM IV criteria [11]. Persons categorized as AD are required to meet NINCDS-ADRDA criteria for possible, probable or definite AD [12]. The diagnosis of vascular dementia (VaD) is made based on ADDTC and NINDS-AIREN criteria, [13] but the presence of vascular dementia does not disqualify a

participant from obtaining a concomitant diagnosis of AD if indicated. Diagnostic criteria for other types of dementia such as Lewy Body disease and frontotemporal dementia are also carefully specified based on published criteria. [14, 15]. We have in the past defined MCI using a purely objective psychometrically-determined definition. However in this paper we use a standard definition of 'probable' MCI based on subjective and/or objective cognitive impairment in one or more cognitive domains with essentially normal functional status (so that the individual did not meet criteria for dementia). For each individual in the dementia surveillance system, a date of onset of cognitive impairment and a date of onset of dementia are assigned based on all available data as described above. Persons meeting criteria for MCI are also categorized as having either amnestic MCI with isolated memory or memory deficits along with other cognitive deficits, and non-amnestic MCI, with either a single or multiple non-memory deficits [16-18].

Neuropathological Assessment

Neuropathological evaluation of all autopsied brains is performed by a single neuropathologist (ACM) who is blinded to all demographic and clinical information. Briefly, the brains are received fresh and the gross neuropathological findings are recorded, including vascular lesions and the degree of atherosclerosis. The length of time between death and receiving the brain and/or placing it on ice varies widely based on whether the death occurred locally or in another state. The median postmortem delay was 6 hours, with the range being 1.5 to 72 hours and the interquartile range varying from 4 hrs (25%th ile) to 14.8 hours (75%th ile). The frontal, temporal and occipital poles are removed from one hemisphere and snap frozen at -80 C. The remaining tissue is fixed in 4% periodate-lysine-paraformaldehyde (PLP) at 4°C for at least 2 weeks. Ten micron paraffin-embedded sections from 30 brain regions, including: the olfactory bulb, 2 levels of the midbrain, 2 levels of pons, medulla, spinal cord, cervical spinal cord and 2 levels of the cerebellum; inferior frontal (Brodmann area (BA) 10,11,12), pre- and post-central (BA 4,3,2,1), inferior parietal (BA 39,40), anterior cingulate (BA 24), superior frontal (BA 9), dorsolateral middle frontal (BA 8), anterior temporal (BA 38), superior temporal (BA 20, 21,22), superior temporal posterior (BA 41,42), posterior cingulate (BA23,31), calcarine (BA 17,18), visual association (BA 19) and superior parietal (BA 7B) cortices; caudate, putamen, and nucleus accumbens (CAP), amygdala, entorhinal cortex (BA 28), the globus pallidus, substantia innominata, anterior hippocampus, hippocampus at the level of the lateral geniculate, and 2 levels of the thalamus are evaluated. Visual association area 19 is defined as the cortical region on the convexity, median and basal surfaces of the cerebrum directly caudal to the parieto-occipital fissure surrounding Brodmann visual area 18, also referred to as the peristriate area.

Histological Stains and Immunohistochemistry

Histological stains includes luxol fast blue, hematoxylin and eosin (LHE), Bielschowsky silver method, and immunocytochemistry for phosphorylated tau protein (Innogenetics, AT8, 1:2000), Aß protein (Dako, 6F-3D, 1:500, pretreated in 90% formic acid for 2 minutes), α- synuclein (Chemicon, affinity purified polyclonal, 1:3000, pretreated in formic acid) and αBcrystallin (Novocastra, NCL-ABCrys, 1:14,000).

Quantitation of Alzheimer's Disease Lesions

Neurofibrillary Tangles—The density of NFT is rated semi-quantitatively in 14 regions using AT8 immunostained sections and in 13 sections using Bielschowsky stained sections. In the neocortical regions, i.e. inferior parietal (BA 40), middle frontal (BA 8), superior temporal (BA 22), calcarine (BA 17) and visual association (BA 19) cortices, a rating of 1+ corresponds to a maximum density of 1 NFT per 200 × field; 2+: 2-5 NFT/field; 3+: 6-9/

field; and 4+: ≥10 NFT/field. For the medial temporal lobe structures, amygdala, entorhinal cortex and hippocampus, NFT are rated as follows: 1+: 1-10 NFT/field; 2+: 11-20 NFT/field; 3+: 21-30/field; 4+ ≥31/field. All determinations are made in areas of maximum involvement at a magnification of 200× using the average count from 3 microscopic fields. For NFT summary scores, the NFT density in the 4 or 5 neocortical areas plus the counts from the 3 medial temporal regions are tabulated. The density of NFT is also evaluated semi-quantitatively in the olfactory bulb, substantia innominata, substantia nigra, locus ceruleus, median raphe nuclei, and dorsal medullary nuclei using AT8 immunostained sections and the same density scale used for the neocortical regions.

Senile Plaques—The density of diffuse (DP) and neuritic (NPL) or compacted senile plaques is determined in the same regions. DP and NPL are rated separately as follows: a score of 1+ corresponds to a density of 1-9 plaques per 100 × microscopic field; 2+: 10-19 per field, 3+: 20-32 per field, and 4+: >32 plaques per field. All determinations are made by averaging the count in 3 microscopic fields in areas of maximum involvement at a magnification of 100×. The 1+ rating corresponds to a CERAD rating of sparse, a 2+ score corresponds to a CERAD rating of moderate, and a 3+ or 4+ score to a CERAD rating of frequent plaques [19]. For a subset of participants, summary scores for DP and NPL in the 7 or 8 most affected regions, as well as for the neocortex and medial temporal lobe structures are also tabulated.

NFT Staging—The distribution of NFT does not always strictly follow the hierarchical pattern described by Braak [20, 21]. Occasionally, modest NFT are found in the medial temporal lobe structures, while equal or greater densities of NFT are found in the neocortical regions. To compensate for non-hierarchical distributions of NFT, a quantitative NFT staging scheme was devised using the numerical sum of NFT density in the same brain regions outlined by Braak. The 6 levels of NFT severity, using AT8 immunostained sections, are defined as follows: Level I: summary score1-4; Level II: score 5-8; Level III: score 9-13; Level IV: score 14-18; Level V: score 19-23; Level VI: score 24-28.

Lewy Bodies—The density of Lewy Bodies is evaluated using α-synuclein immunostained sections of the olfactory bulb, 2 levels of the substantia nigra, inferior parietal cortex, anterior cingulate cortex, middle frontal cortex, superior temporal cortex, amygdala, entorhinal cortex, transentorhinal cortex, substantia innominata, hippocampus, locus ceruleus, median raphe nuclei and dorsal medullary nuclei.

Argyrophilic Grains—For the presence of argyrophilic grain disease, silver and AT8-positive medial temporal lobe (amygdala, entorhinal cortex and hippocampus) grains and αBcrystallin-positive ballooned neurons in the amygdala and entorhinal cortex are required.

Vascular and Microvascular Lesions

The FHS has been systematically documenting measures of vascular pathology and has developed a composite measure of vascular pathology derived using the National Alzheimer Co-ordinating Center (NACC) and University of California at Los Angeles (UCLA) ischemia score protocols and consistent with the Vascular Cognitive Impairment Harmonization guidelines [22]. The developed ischemic injury scale (IIS) includes assessments of hippocampal sclerosis, volume of chronic infarcts, number of lacunes and microinfarcts, degree of atherosclerosis, arteriolosclerosis and white matter disease (including white matter integrity) and gives an overall single IIS score for each brain. Hippocampal sclerosis is judged by the presence of neuronal loss and gliosis in the hippocampal CA fields and subiculum using the following semi-quantitative scale: 0=none; 1=CA1 only; 2=CA1/Subiculum; 3=Subiculum fields/Prosubiculum; 4= All CA fields,

subiculum/Prosubiculum. 'Microinfarcts' are defined as encephalomalacic lesions, 2 mm or smaller in greatest dimension, not identifiable on gross inspection of the brain. They are located in the cortex and subcortical white matter and include cavitated and non-cavitated chronic microinfarcts and microhemorrhages. Cavitated microinfarcts are defined as cystic areas of tissue loss or collapse with gliosis, and usually, macrophage infiltration. Non-cavitated microinfarcts are focal areas of cellular loss and gliosis without the formation of a cystic cavity. Microscopic deposits of blood or hemosiderin with minimal evidence of ischemic infarction are designated as microhemorrhages. The number of microinfarcts and microhemorrhages are tabulated in 5 neocortical regions and underlying white matter, hippocampus, entorhinal cortex, brainstem, and deep nuclei, including caudate, putamen, globus pallidus, thalamus, and amygdala. The number of microinfarcts and microhemorrhages are then recorded as a semiquantiative score per region: 0 = no microinfarcts; 1+ = 1-3 microinfarcts; 2+ = 4-8 microinfarcts; 3+ = 9-19 microinfarcts; 4+ = ≥20 microinfarcts. Degree of arteriolosclerosis is a composite score of the degree of hyaline thickening of arteriolar walls evaluated semi-quantitatively in 4 regions of deep white matter and basal ganglia. White matter disease is judged using a summary score of myelin loss and cribriform state evaluated in the subcortical white matter of 4 regions and the basal ganglia. Myelin loss is judged by gross inspection of the luxol fast blue, hematoxylin and eosin stained slide and rated semiquantitatively. If the area to be evaluated contains an infarct, the area is omitted from the analysis. Cribriform state is judged as a summary score of the following: 1. Perivascular rarefaction - the degree to which the tissue is attenuated or vacuolated around small blood vessels. 2. Dilation of perivascular spaces -widening of the perivascular space, using the greatest degree of dilation found around a single vessel seen in cross-section. 3. Perivascular macrophages- no perivascular macrophages = 0, 1-3 macrophages around a single small vessel = 1+, 4-8 macrophages = 2+, ≥ 9 macrophages = 3+. In addition to the measures that comprise the IIS, amyloid angiopathy is also evaluated in the leptomeninges and in the parenchyma in 4 neocortical regions: middle frontal, inferior parietal, superior temporal and calcarine cortices using a scale of severity modified from Von Sattel *et al.*[23] and Esiri *et al.*[24]

Project 1: Diagnostic Accuracy of a Clinical Alzheimer's Disease Diagnosis in the Framingham Heart Study

Rationale

Histopathological examination of the brain remains the only method for determining definite AD in an individual patient. However, an AD diagnosis can be made in life with excellent sensitivity (between 80% and 100%) in most specialized centers [9, 25-27], even in mildly affected individuals [28]. The largest study comparing the clinical diagnosis of AD with the neuropathologic findings in specialized centers found the clinical diagnosis of AD to be correct in 93% [27]. The specificity of the clinical diagnosis at these centers was somewhat lower at 55%, but most of the cases incorrectly diagnosed as AD in this study had equally irreversible degenerative dementias. In the clinical diagnosis of AD, high sensitivity may be more important than high specificity, as long as clinicians do not incorrectly diagnose and miss the opportunity to treat reversible disorders.

Methods and Results

To examine the accuracy with which AD is diagnosed in the FHS, we compared the clinical diagnoses and blinded neuropathological diagnoses of AD in 92 successfully autopsied FHS subjects. Among 92 brains collected and autopsied between 1997 and 2002, 27 of 36 subjects with pathologically verified AD were clinically diagnosed with AD (75.0% sensitivity), while 52 of 56 subjects without AD were correctly identified as not having AD (92.9% specificity). Among these 92 FHS brains, 59 were from subjects who had been

examined within 2 years of death. Of these, 17 of 22 subjects with pathologically verified AD were clinically diagnosed with AD (77.3% sensitivity), while 34 of 37 subjects without AD were correctly identified as not having AD (91.9% specificity). The five cases that met neuropathological criteria for AD and were not clinically identified as AD were, however, all noted to be cognitively impaired in their final clinical evaluations. Two of the five cognitively impaired subjects were incorrectly diagnosed in life with Lewy Body Dementia rather than AD.

Discussion

These results indicate that the clinical diagnosis of AD is reasonably accurate among FHS participants who come to brain autopsy. The accuracy of diagnostic assessments in the FHS is not surprising given the long-term effort that is made to examine and evaluate participants. The 92 participants in this series had been followed with regular neurological and neuropsychological evaluations. Furthermore, unlike AD research centers where unusual cases may be sent for referral, the FHS is a community-based study and thus far, there are relatively few dementia diagnoses beyond AD and vascular dementia.

Diagnostic accuracy for AD in community-based studies has been previously reported as 71% in a community survey in Finland, [29] 63% in a screening study in Japan, [30] 65% in the Honolulu-Asia Aging Study [31] and 84-93% in a preliminary report from the Religious Orders Study [32]. The overall accuracy of the clinical diagnosis of AD in Framingham subjects who came to autopsy (73.1%) is consistent with these reports, most of which are less accurate than the accuracy of AD diagnosis in specialized centers where smaller numbers of subjects can be followed closely in the years prior to death. Indeed, among Framingham subjects who were examined by either a neurologist or neuropsychologist within 2 years of their death, the sensitivity of the diagnosis was 83.3%, much closer to the numbers obtained in specialized clinics.

The specificity of the diagnosis of AD is high among Framingham participants in comparison to that in specialized centers, probably because non-AD dementias are concentrated in specialized referral centers and are more likely to be misdiagnosed as AD. Our report is limited by this relative homogeneity of diagnostic categories, as well as a small sample size. It is also possible that those participants volunteering for brain donation may be different from those who did not volunteer. However, in comparison to neuropathology, the low number of misclassifications by clinical evaluation adds confidence to epidemiologic inferences based upon clinical determinations of AD.

Project 2: Neuropathological Correlates of Cognitive Impairment Associated with Alzheimer-Type Pathological Processes in the Framingham Study

Rationale

In previous studies, the extent of neuropathological changes of Alzheimer disease (AD) at post-mortem examination has shown a poor correlation with cognitive status in as many as 30% of prospectively studied older individuals (above age 75 years). Some cognitively normal subjects are reported to have neuropathological findings diagnostic of AD, [33, 34] while a few demented individuals clinically suspected to have AD show no pathological changes of AD. This discordance between clinical and neuropathological findings has been hypothesized to increase with increasing chronological age[35-37].

In an effort to further define the neuropathological correlates of poor cognitive status in a population-based sample of persons presumed to have different stages of a largely

Alzheimer-type pathology, we analyzed these clinicopathological associations in a FHS subsample. In this analysis, we broadened our regional neuropathological analysis of AD pathology to include diverse locations including the olfactory bulb, nucleus basalis of Meynert, substantia nigra, pons and medulla, in addition to the standard regions typically examined in such studies, the frontal, parietal, temporal, occipital, and entorhinal cortices, amygdala, and hippocampus.

Study Sample and Methods

We included those subjects who came to autopsy between 1997 and 2003, and were 75 years or older at the time of death (n=42); at the time of death these subjects were determined to have a retrospective clinical dementia rating (CDR) ranging from 0.0 to 3.0. If a subject was considered mildly impaired (CDR 0.5), a determination was made as to whether the impairment included a substantial memory component or not. Only subjects considered CDR 0.5 on the basis of a memory impairment were included in this analysis since it is believed that non-amnestic MCI is more likely to represent an early vascular cognitive impairment. Subjects with dementia (CDR 1.0-3.0) were further restricted to those with a clinical diagnosis of AD; subjects clinically diagnosed with a dementia not related to AD, including all persons with a clinical stroke and/or probable vascular dementia by NINDS-AIREN criteria, were excluded from the analysis.

A total of 42 subjects aged 75 years and older came to autopsy in this time period, and of these, 8 subjects were excluded on the basis of confounding pathology (e.g., progressive supranuclear palsy, frontotemporal dementia, multiple infarcts, lacunes, or multiple traumatic cerebral contusions). Thirty-four remaining subjects, ranging in age from 75.2 to 99.7 years (mean 89 ± 6.2 years), 21 women and 13 men, were the focus of this analysis. Of the 34 subjects, 31 subjects were from the Gen 1 cohort and 3 subjects were from the Gen 2 cohort. Eighteen of the 34 subjects (52.9%), were considered cognitively intact at the time of death with CDR scores of 0.0, 3 subjects were mildly impaired with CDR scores of 0.5 (8.8%), and 13 were demented with CDR scores of 1.0 to 3.0 (38.2%). Fourteen subjects (42%) held at least one college degree, 25% had at least some education beyond high school, 18% had graduated from high school, 12% had some high school education, and one subject (3%) had only completed elementary school. The age-, sex- ratio, educational levels, and proportion of the sample with at least one ApoE ε4 allele was not significantly different among the clinical groups (Table 1).

Statistical Analyses

We summarized the distributions of variables of interest using box plots, Fig. (1) (with the top edge of each box at the 75th percentile, the bottom edge of the box at the 25th percentile, and the thick line in the interior of the box representing the median). The neuropathological variables used in this study were found to not follow Gaussian distributions. As a consequence, we employed non-parametric statistical procedures including the Spearman rank correlation, theWilcoxon rank sum test (in order to compare two groups), and the Kruskal-Wallis test (a non-parametric analogue to analysis-of-variance). For analyses of categorical variables, we constructed cross-tabulations and employed Fisher's exact tests of hypothesis. Although the analyses are exploratory we corrected for multiple testing and determined that the results from these analyses were statistically significant if p was less than or equal to 0.005.

Results

The correlation of individual neuropathological markers and of specific diagnoses with clinical and functional status as determined by the Clinical Dementia Rating (CDR) Scale score is described below.

Correlation of Neuropathological Markers with CDR Category

Neurofibrillary Tangles—In all clinical groups, NFT were most dense in the entorhinal cortex, hippocampus and amygdala. Within the neocortex, NFT were most dense in the superior temporal cortex, followed by the inferior parietal and dorsolateral frontal cortices, whereas NFT were least frequently found in the primary visual cortex, area 17. NFT density measurements obtained with Bielschowsky silver staining were generally lower than those obtained with AT8 immunostaining, although the same relationships were identified. The summary score for overall NFT, whether identified with AT8 immunostain or Bielschowsky silver stain, rose with increasing antemortem CDR score (Fig. 1, Table 2). Neocortical NFT also rose with dementia severity in all neocortical regions whether considered individually or as a group. However, there was a significant difference in NFT density between persons with a CDR of 0.0 and those with a CDR of either 0.5 or CDR 1.0 only in the dorsolateral frontal cortex. (Post hoc test of pairs of groups via Wilcoxon Rank Sum procedure, AT8 NFT, 0 vs 0.5, p =.005; 0 vs 1, p = .004; 0 vs. 0.5 and 1 combined, p = .0003, Bielschowsky NFT, 0 vs 0.5, p =.0006; 0 vs 1, p = .0003, (Table 2). In the cognitively unimpaired (CDR 0.0) subjects, scattered NFT were common in superior temporal cortex and, to a lesser extent in the inferior parietal cortex. NFT in the dorsolateral frontal cortex were unusual in the CDR 0.0 subjects. CDR 0.0 subjects also had significantly fewer overall, neocortical, inferior parietal, dorsolateral frontal, and superior temporal NFT when compared to persons with CDR 0.5 or higher.

Medial temporal lobe NFT density increased with rising CDR scores, although the differences between the groups were not significant. There was a significant difference between CDR 0.0 (unimpaired) and CDR 0.5 plus 1.0 subjects restricted to the amygdala (Table 2). AT8 immunopositive and Bielschowsy silver stained NFT were present in the CA1 region of the hippocampus in every subject, although they were rare in 3 unimpaired subjects. Seven unimpaired subjects (38.9%) had abundant hippocampal NFT, and 6 unimpaired subjects (33%) had numerous entorhinal tangles. NFT were also found in the CA2 region of the hippocampus in nearly all subjects, they were not found in the 3 unimpaired subjects with only rare NFT in CA1. The density of NFT in the CA1 and CA2 was not correlated with age. Moreover, CA2 NFT density did not correlate with CA1 NFT density.

Occasional AT8 immunopositive NFT were found in the in the nucleus basalis of Meynert in all CDR 0.0 subjects, 83.3% of the cognitively unimpaired, CDR 0.0, subjects had at least some NFT in the olfactory bulb, 77.7% had NFT in the locus ceruleus and raphe nuclei, and 22.7% had NFT in the substantia nigra. In the substantia nigra, NFT were significantly increased in the CDR 0.5 or 1.0 group and in the CDR 1.0 subjects compared to the CDR 0.0 subjects. There was also a tendency toward increasing NFT density with increasing severity of dementia in the nucleus basalis of Meynert (Table 2).

Senile Plaques—Of the 18 CDR 0.0 subjects, 3 (16.7%) had no senile plaques, diffuse or neuritic, as determined by Aß immunostaining and Bielschowsky silver stain. Four CDR 0.0 subjects (22.2%) had only sparse diffuse plaques (DP), and 11 subjects (61.1%) had moderate DP plus sparse to moderate NPL. All impaired subjects (CDR 0.5) had at least moderate densities of DP and NPL. Diffuse plaques were most frequent in dorsolateral frontal cortex, followed by the superior temporal and inferior parietal cortex. The total number of DP did not distinguish CDR 0.0 subjects from mildly impaired subjects (CDR 0.5), but there was a significant difference in DP when the CDR 0.0 group was compared to the CDR 0.5 plus 1.0 (p = .004), and CDR 3.0 (p = .003) groups (Table 2).

Neuritic plaques were most frequent in the dorsolateral frontal cortex, followed by the superior temporal cortex, inferior parietal cortex and amygdala. Overall NPL scores did not

significantly distinguish CDR 0.0 subjects from mildly impaired (CDR 0.5) subjects, although there was a significant difference between CDR 0.0 and CDR 0.5 plus CDR 1.0 ($p = .003$), CDR 2.0 ($p = .005$), and CDR 3.0 ($p = .0009$) subjects. Diffuse and NPL density increased to a plateau with mild cognitive impairment, increasing only minimally with further cognitive impairment Fig. (1).

Prevalence of Pathological AD in this Subsample Using Different Criteria

The Consortium to Establish a Registry for Alzhemier's Disease (CERAD) Criteria: [19, 38]—Of the 18 CDR 0.0 cases, 10 had moderate or frequent NPL in the neocortex and would fall into the category of "Possible AD" by CERAD criteria, as there had been no clinical evidence of dementia. The 3 CDR 0.5 AD cases would also fall into the category of "Possible AD" based on the absence of overt clinical dementia and the presence of at least moderate NPL. Three of the 13 CDR ≥ 1 AD subjects would be classified as "Probable AD", and 10 would be classified as "Definite AD" using CERAD guidelines.

NFT Stage-Based Criteria—Five of the 34 cases (14.7%) had non-Braak patterns of NFT deposition, typically consisting of cortical neurofibrillary degeneration without dense medial temporal lobe NFT. However, both the semi-quantitative numerical NFT staging system and the Braak hierarchical NFT staging system gave comparable results Fig. (2). NFT staging was significantly different among the CDR groups using either scheme (Fisher's exact test, $p < .0001$).

National Institute on Aging (NIA)-Reagan Criteria—Six of the 18 CDR 0.0 subjects (33%) did not meet NIA-Reagan criteria for AD as there were no NPL. Six others had a low likelihood of AD (33%) based on a low density of neocortical NFT (Braak stage I-II) and/or NPL (sparse). Six other CDR 0.0 subjects (33%) had an intermediate likelihood of AD based on moderate densities of NPL and NFT stage III (28%) or IV (5%). Two CDR 0.5 AD subject were classified as having an intermediate likelihood of AD (33%) and one was classified as high likelihood of AD (67%). Among the 13 subjects with CDR ≥ 1.0, one had an intermediate likelihood of AD with a NFT stage IV, the other 12 were classified as high likelihood of AD.

In summary, in our sample the density and distribution of NFT showed a better congruence with antemortem clinical diagnosis and cognitive status than the CERAD or NIA-Reagan criteria for pathologically determining the likelihood of Alzheimer's disease.

Prevalence of Alternative Neuropathological Diagnoses in this Older, Clinical AD Subsample

Lewy Bodies—Isolated α-synuclein positive Lewy bodies were found in the substantia nigra in 2 CDR 0.0 subjects (11.1%).

Argyrophilic Grain Disease—Five (27.8%) cognitively normal subjects had argyrophilic grains and αBcrystallin-positive ballooned neurons in the amygdala and entorhinal cortex, as well as 2 of the 3 CDR 0.5 subjects (67.7%), and 6 (46.2%) of the subjects with CDR 1.0-3.0.

Discussion

Our results demonstrate that among elderly FHS subjects who are either normal or have amnestic mild cognitive impairment or probable AD, and who have been clinically followed for decades, there is close agreement between the mental status evaluation during life and neuropathological findings of AD at autopsy. We found a significant increase in overall,

neocortical, frontal, parietal, temporal, and amygdala NFT in CDR 0.5 plus 1.0, CDR 2.0, and CDR 3.0 subjects compared to cognitively intact, CDR 0.0, subjects. Moreover, the same relationships were significant whether NFT were identified with phosphorylated tau protein (AT8) or Bielschowsky silver stain. These findings are consistent with previous reports correlating neocortical NFT density and cognitive impairment in severely demented individuals [39, 40] and extend the correlation to mildly impaired and mildly demented subjects. Our findings are in contrast to prior reports of clinical mild cognitive impairment in individuals with no cortical NFT and low Braak stages, [41] and reports that Braak staging did not distinguish elderly individuals with normal cognition from those with CDR 0.5 [42]. This difference might be partly attributable to the fact that we excluded persons with non-amnestic MCI.

Remarkably, of the 4 neocortical regions examined, only the density of NFT in the dorsolateral frontal cortex distinguished subjects with MCI (CDR 0.5), from those considered cognitively intact (CDR 0.0). NFT in the dorsolateral frontal cortex also uniquely distinguished the mildly demented, CDR 1.0, subjects from the CDR 0.0 subjects. Thus presence of scattered NFT in dorsolateral frontal cortex, when combined with more widespread neocortical neurofibrillary pathology was associated with both MCI and mild dementia. The correlation of frontal NFT with clinically perceptible cognitive deficits might be a reflection of overall pathological severity, i.e. neocortical neurofibrillary pathology sufficiently severe and widespread to produce clinical symptoms. On the other hand, the involvement of the dorsolateral frontal cortex, which would be expected to result in dysexecutive symptoms and frontally-mediated behavioral disturbances, could explain early executive function deficits often seen in population based samples of MCI and early AD. We also know from studies of persons with AD, that executive cognitive dysfunction and frontal behavioral disturbances are frequently observed and are associated with functional impairment in activities of daily living [43, 44]. Furthermore, there is evidence that frontally-mediated behaviors and executive dysfunction are common in subjects with mild cognitive impairment, even before functional decline in daily living is evident [45].

In addition to a significant increase in dorsolateral frontal NFT, substantia nigra NFT were significantly increased in CDR 0.5 plus CDR 1.0 subjects compared to CDR 0.0 subjects. It is noteworthy that lesions of the substantia nigra have also been associated with cognitive disturbances [46, 47].

We found that 16.7% of the 18 CDR 0.0 subjects had no Aß deposition, either as vascular amyloid, diffuse or neuritic plaques. Another 22.2% of the unimpaired subjects had only rare DP in the neocortex. Isolated neocortical NFT were found in these unimpaired subjects with little or no amyloid deposition, suggesting the hierarchical pattern of NFT deposition in the elderly is characterized by scattered neocortical NFT even in the absence of Aß.

Additionally, there was a distinct rise in DP and NPL plaque density in subjects with CDR 0.5 compared to CDR 0.0 subjects, but only mild increases with further increments in CDR. In contrast, overall NFT density showed progressive increases as CDR scores climbed from 0.0 to 0.5 and higher. These findings are strikingly similar to the observations of Ingelsson and colleagues regarding the time course of pathological changes within the temporal cortex in AD cases of varying disease duration [48]. Our findings support the concept that Aß deposition rises to an early plateau in mildly impaired and demented subjects without substantial increases as dementia severity worsens, yet NFT formation shows an incremental rise with increasing cognitive decline and continues to progress throughout all levels of dementia severity.

We did not find any instance of ‘dementia of unknown etiology’ among the 34 participants, despite a mean age of 89 years. Whether the exclusionary criteria for the analyses, which eliminated subjects with cognitive decline considered secondary to a vascular event, accounts for this discrepancy is unknown. Among the cases used for this specific analysis (a subset of all persons who have had autopsy), we also found no subjects with substantial Lewy Body pathology to support a diagnosis of Lewy Body disease or Parkinson's disease despite extensive histopathological scrutiny. Argyrophilic grain disease was found in a sizeable percentage of cognitively normal subjects (28%) and a larger percentage of impaired subjects (46-67%), consistent with previous reports [33, 49].

Among elderly subjects in the Framingham study, clinically followed for 29 - 50 years, there is a detectable rise in cerebrocortical Alzheimer pathology as cognitive ability deteriorates. Cognitively intact CDR 0.0 subjects can be distinguished from mildly impaired CDR 0.5 subjects on the basis of silver or tau positive NFT in the dorsolateral frontal cortex, and if the mildly demented CDR 1.0 subjects are included, by overall NFT density, DP and NPL density, as well as by the density of NFT in the neocortex and substantia nigra. The strength of our analyses lie in the community-based sample and longitudinal, intensive, antemortem examination protocols.

Project 3: Ischemic Injury Score (IIS): Correlation with Antemortem CDR Score in all Persons, and in Persons with MCI

Rationale

Today, the diagnosis of MCI made in a memory clinic is often considered to represent a prodromal clinical stage of Alzheimer's disease (AD)[50-52]. Neuropathological evidence suggests that in clinic-based series, at the time a clinical diagnosis of MCI is made, AD pathology is already present [50, 53-57]. In fact, it appears that the hallmark signs of tau and amyloid pathology may develop decades before the detection of clinical impairment as evidenced by the repeated observation that brains of cognitively normal elderly individuals have mild to moderate degrees of AD pathology [33, 34, 36]. Further, studies of young Down syndrome brains indicate that Aß deposition and NFT degeneration precede the development of clinical symptoms of AD (progressive cognitive deterioration from young adult baseline) by 10 to 20 years [58].

Thus, often by the time an individual is diagnosed with MCI, AD pathology is fully developed [50, 59], unless the cognitive impairment is due to mixed pathologies or there is a second contributing pathological process [41, 51, 53, 54, 60, 61]. Clinical and pathological studies suggest that concomitant vascular disease is common and determines the time of onset of clinical symptoms in persons with Alzheimer's disease pathology [62-66]. Such overlapping pathology is also likely in persons with MCI. Indeed, antemortem evidence of vascular pathology (e.g., WMH, MRI infarcts) in amnestic and non-amnestic MCI has been demonstrated through imaging studies [11, 67]. Post-mortem studies, however, have also emphasized the importance of medial temporal lobe AD pathology in persons with amnestic MCI [61], and there are published reports that 20-50% of persons with non-amnestic MCI also have diagnostic AD pathology at autopsy [61]. We predicted that subjects with MCI would have significantly more ischemic pathology than cognitively intact subjects. We also predicted that vascular pathology would act additively with NFTs to produce cognitive decline, but that NFTs would be the strongest overall predictor of impaired cognitive status.

Study Samples, Methods and Results

We examined the association between the IIS and CDR category assigned at post-mortem in 103 FHS subjects who came to autopsy between 1997 and 2008. We compared the mean

summary ischemic scores across CDR categories using one-way analysis of variance with additional multiple comparisons made using Tukey's test, using an alpha of 0.05 to denote statistical significance. The mean ischemic score in this sample was 5.8 (standard deviation=3.9; minimum=0, 25th%ile=3, median=6, 75th%ile=9, maximum=21). In a subset of 18 brain donors who had MCI at the examination immediately preceding their death, 10 of whom had amnestic-MCI, we examined NFT and NPL burdens as well as the IIS and compared the findings with those in cognitively normal persons (CDR 0).

Results showed an overall difference in mean summary ischemic score across CDR categories, ($p = 0.03$), with highest values noted in persons with a CDR of 0.5. The sole comparison that was statistically significant by Tukey's test was between CDR group 0 and CDR group 0.5. Analysis of component scores showed significant results for Chronic lacunes, $p = 0.02$ and Atherosclerosis, ($p = 0.04$). Among the subgroup of persons with either MCI or normal cognition, persons with any (amnestic or non-amnestic) MCI did not differ significantly from persons with a CDR=0 in NFT or NPL burden, but were more likely to have lacunes, perivascular rarefaction and severe arteriosclerosis as well a higher summary IIS score ($p<0.05$), emphasizing the contribution of vascular pathology to MCI in a community-based sample of the elderly. However, comparing persons with clinical *amnestic* MCI (with CDR 0.5) to cognitively normal persons, we observed that the former had significantly higher burdens of NFT, NPL and amyloid angiopathy ($p<0.01$). As more cases accrue, we will re-examine the stability of these preliminary findings.

Discussion

The importance of concomitant vascular pathology in determining the severity of clinical manifestations in persons with dementia due to Alzheimer's disease has been clearly shown in landmark papers from the Religious Orders Study [68, 69], the Honolulu-Asia-Aging Study [70, 71] and the Medical Research Council Cognitive Function and Aging Study[72]. There is less information on the contribution of vascular pathology to mild cognitive impairment, early studies suggested that there was an independent additive effect of vascular and AD pathology, [73] although recently the contribution of microinfarcts in the absence of macroscopic infarcts has been questioned [74].

In our analysis using CDR category as dependent variable and both the IIS and markers of AD pathology as the independent variables, we observed that the greater the degree of tangles observed, there was a significantly greater likelihood of a worse CDR. Moreover, neither IIS nor plaque loads were independently significantly associated with poorer CDR. Whereas ischemic injury does have a significant negative impact on cognitive function, this impact is largely in the early stages of MCI. As NFT burden (and concomitant cognitive impairment) increases, the relative contribution of vascular injury appears to be attenuated.

Concluding Remarks and Future Plans

FHS' neuropathological program strengthens and validates its clinical and genetic studies. The diagnostic sensitivity and specificity analyses presented here suggest that the FHS' accuracy in clinical diagnosis is comparable to that of other epidemiologic studies and Alzheimer's Disease Center programs. A strength of FHS' neuropathological investigation is that it derives from a multi-generational community-based cohort, and has autopsy cases that reflect the full-spectrum of cognitive function from cognitively intact to severe impairment. Our clinico-pathological analyses, although they should be considered exploratory requiring confirmation in other datasets, suggest that despite the significant burden of vascular risk/disease in persons with AD, it is the NFT burden that correlates most strongly with clinical and functional status. However, our findings that vascular injury plays a role in the earliest, MCI stages of AD suggests that reducing risk factors that lead to pathological vascular insult

may help delay disease onset. In prior publications FHS brain autopsy data has been used to propose novel hypothesis, for example, (i) that dense neurofibrillary tangles (NFTs), and neuritic plaques (NPls) in the visual (parietal) association cortex Brodmann area 19 may be observed in the absence of substantial pathology in the hippocampus or entorhinal cortex [75], and (ii) that there may be increased expression of receptors for brain-derived neurotrophic factors in hippocampal CA1 neurons during mid-stage Alzheimer disease [76].

With aging of the Gen 2 cohort, the FHS' neuropathological program will continue to be representative of a community-based sample and should allow further follow-up and extension of current investigations. Further, there will be increasing numbers of parent-offspring dyads that will open up novel opportunities for familial studies of pathology. Our plans for the coming 2-3 years include using neuropathology to explore correlations with antemortem imaging, as a sensitive endopheotype, and using brain tissue for studies of genomics, gene expression and epigenetic modifications. Some advantages of the FHS brain bank are the availability of genome-wide genetic and a wide range of biomarker data permitting the comparison of blood and brain expression. Further the lifestyle and risk factor data gathered over the lifetine of the brain donors can be related to gene expression and the observed neuropathology. Specific ongoing projects include (i) using pathological endophenotypes to improve sensitivity in identifying genetic and environmental risk factors for Alzheimer's disease and vascular brain injury, (ii) using neuropathology to explore pathophysiologic pathways between risk factors and clinical disease (iv) undertaking gene-specific and agnostic expression analyses and epigenetic studies on specific brain regions and in specific cell types.

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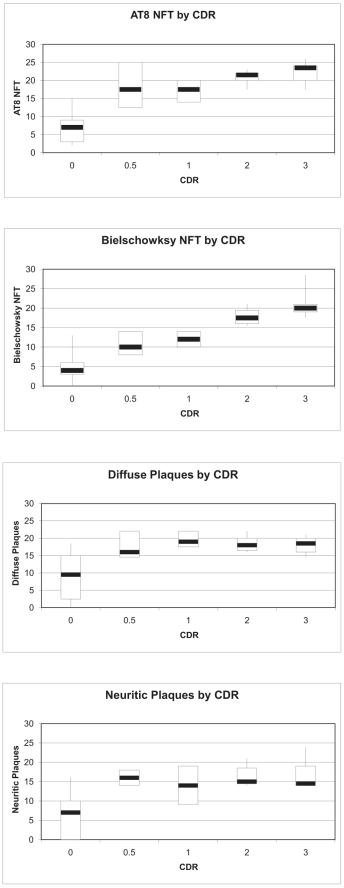

Fig. (1).
Box plots of distribution of neuropathology parameters by Clinical Dementia Rating Scale (CDR) score (n = 34).

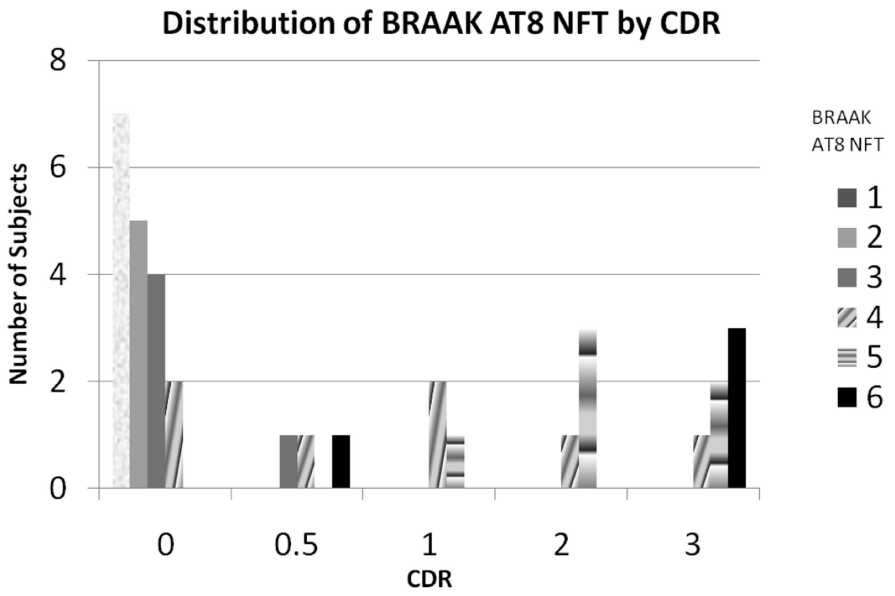

Fig. (2).
Bar chart of Braak neurofibrillary tangle (NFT) stage by Clinical Dementia Rating Scale (CDR) score.

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Table 1

Demographic Characteristics, Clinical Data and ApoE Genotype of Subjects

CDR Group	0	0.5	1	2	3
Number of Subjects (n= 34)	18	3	3	4	6
Mean Age at Death (± SD), y	88.6±6.4	86.5±5.4	90.7±7.4	86.1±4.1	92.3±2.5
Sex F/M	10/8	3/0	2/1	2/2	4/2
ApoE genotype (n=27)					
23	1	0	0	0	0
24	1	1	0	0	0
33	11	2	1	2	1
34	2	0	1	1	1
44	0	0	0	0	1
% with 1 or 2 apoE 4	6.7%	0%	50%	33%	67%
Education Level (n=33)					
Elementary	1	0	0	0	0
Some High School	2	0	0	2	0
High School Graduate	3	0	0	1	2
Any College	11	3	3	1	4

Table 2

Statistical Comparison of Distribution of Neuropathology Parameters by CDR (N=34)

Table 2.	CDR					P-Values:	
	0	0.5	1	2	3	Overall	
	(N=18)	(N=3)	(N=3)	(N=4)	(N=6)	0 vs 0.5	0 vs 0.5, 1
						0 vs 1	0 vs. 2
						0.5 vs 1	0 vs. 3
	Median (25th, 75th percentiles)						
Summary-AT8 NFT	6.5 (3,9)	18 (12, 25)	18 (14, 20)	22 (20, 22.5)	23.5 (20, 24)	<.0001	
						0.02	0.001
						0.01	0.002
						1	0.0004
Cortical-AT8 NFT	1.0 (0, 3)	6 (4, 13)	8 (5, 12)	10.5 (10, 11)	12.5 (9, 16)	<.0001	
						0.01	0.0007
						0.01	0.002
						1	0.0003
Dorsolateral Frontal- AT8 NFT	0 (0, 0)	2 (1, 3)	4 (1, 4)	3.5 (2.5, 4)	3 (2, 4)	<.0001	
						0.005	0.0003
						0.004	0.0005
						0.5	<.0001
Inferior Parietal- AT8 NFT	0 (0, 1)	2 (1, 3)	2 (1, 4)	3 (3, 3.5)	3.5 (3, 4)	<.0001	
						0.01	0.001
						0.01	0.0007
						1	0.0001
Superior Temporal- AT8 NFT	1 (0, 2)	2 (2, 4)	2 (2, 4)	4 (4, 4)	4 (3, 4)	<.0001	
						0.04	0.005
						0.04	0.002
						1	0.0002
Calcarine- AT8 NFT	0 (0, 0)	0 (0, 3)	0 (0, 1)	0 (0, 0)	3 (1, 4)	0.0004	
						0.02	0.02

Table 2.	CDR					P-Values:	
						0.02	0.01
						1	<.0001
Medial Temporal-AT8 NFT	4.5 (3, 6)	8 (6, 12)	9 (6, 12)	12 (9.5, 12)	10.5 (9, 11)	0.003	
						0.03	0.009
						0.08	0.01
						0.64	0.005
Hippocampus- AT8 NFT	2 (1, 3)	4 (1, 4)	3 (2, 4)	4 (2.5, 4)	3.5 (3, 4)	0.09	
						0.03	0.03
						0.24	0.15
						0.2	0.16
Entorhinal- AT8 NFT	1 (1, 3)	3 (2, 4)	3 (2, 4)	4 (3, 4)	4 (3, 4)	0.005	
						0.04	0.01
						0.08	0.02
						0.81	0.003
Amygdala- AT8 NFT	1 (1, 2)	2 (2, 4)	3 (2, 4)	4 (4, 4)	4 (3, 4)	0.0009	
						0.03	0.005
						0.04	0.004
						0.81	0.002
Olfactory Bulb- AT8 NFT	1 (1, 2)	2 (2, 4)	2 (1, 2)	3.5 (3, 4)	4 (2.5, 4)	0.052	
						0.07	0.08
						0.39	0.07
						0.3	0.04
Substantia Nigra- AT8 NFT	0 (0, 0)	1 (1, 3)	2 (2, 4)	2 (1, 2)	2 (2, 3)	0.0004	
						0.01	0.0007
						0.003	0.03
						0.37	0.0002
Nucleus Basalis- AT8 NFT	2 (1, 2)	3 (2, 4)	3 (2, 3)	4 (3, 4)	4 (4, 4)	0.0006	
						0.04	0.007
						0.0497	0.008

Table 2.	CDR					P-Values:	
						0.81	0.0007
Raphe- AT8 NFT	1 (1, 2)	2 (1, 3)	2 (2, 3)	4 (3.5, 4)	3 (2, 3)	0.001	
						0.23	0.03
						0.0467	0.002
						0.81	0.005
Locus Ceruleus- AT8 NFT	1 (1, 2)	2 (2, 4)	2 (0, 3)	3 (3, 4)	3 (3, 3)	0.006	
						0.1	0.16
						0.7	0.01
						0.64	0.003
Summary- Bielschowsky NFT	3.5 (2, 6)	10 (8, 14)	11 (10, 14)	17 (15.5, 19.5)	20 (19, 21)	<.0001	
						0.049	0.006
						0.03	0.002
						0.65	0.0003
Cortical- Bielschowsky NFT	0 (0, 1)	4 (2, 5)	5 (4, 6)	8 (7, 8.5)	10 (8, 13)	<.0001	
						0.01	0.0009
						0.007	0.001
						0.37	0.0002
Dorsolateral Frontal-	0 (0, 0)	1 (1, 1)	2 (1, 2)	2.5 (1.5, 3.5)	2.5 (1, 4)	<.0001	
Bielschowsky NFT						0.0006	<.0001
						0.0003	<.0001
						0.19	<.0001
Medial Temporal-	3 (2, 5)	8 (4, 9)	6 (4, 10)	10 (7.5, 12)	11 (10, 12)	0.003	
Bielschowsky NFT						0.15	0.051
						0.14	0.01
						1	0.002
Summary- Diffuse Plaques	9.5 (2, 15)	16 (14, 22)	19 (17, 22)	17.5 (16.5, 20)	18.5 (16, 20)	0.001	
						0.06	0.004
						0.01	0.01
						0.51	0.003

Table 2.	CDR					P-Values:	
Summary- Neuritic Plaques	7 (0, 10)	16 (14, 18)	14 (9, 19)	15.5 (14.5, 18.5)	15 (14, 19)	0.0004	
						0.01	0.003
						0.054	0.005
						0.82	0.0009
Summary- All Plaques	15.5 (2, 25)	32 (28, 40)	31 (28, 41)	34.5 (31, 38.5)	33.5 (30, 39)	0.0002	
						0.01	0.0009
						0.01	0.004
						1	0.0007