Published in final edited form as:

Neurobiol Aging. 2016 January ; 37: 1–11. doi:10.1016/j.neurobiolaging.2015.10.009.

Brain pathologies in extreme old age

Janna H. Neltner, MD¹, Erin L. Abner, MPH^{5,6}, Gregory A. Jicha, MD, PhD^{2,5}, Frederick A. Schmitt, PhD^{2,5}, Ela Patel⁶, Leonard W. Poon, PhD⁸, Marla Gearing, PhD⁹, Robert C. Green, MD, MPH¹⁰, Adam Davey¹³, Mary Ann Johnson⁸, S. Michal Jazwinski, PhD¹⁴, Sangkyu Kim, PhD¹⁴, Daron Davis, MD¹², John L. Woodard, PhD¹¹, Richard J. Kryscio, PhD^{3,6}, Linda J. Van Eldik, PhD^{4,6}, and Peter T. Nelson, MD, PhD^{1,6,*}

¹Department of Pathology, Division of Neuropathology, University of Kentucky, Lexington, KY

²Department of Neurology, University of Kentucky, Lexington, KY

³Department of Statistics, University of Kentucky, Lexington, KY

⁴Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY

⁵Department of Epidemiology, University of Kentucky, Lexington, KY

⁶Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY

⁷Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN

⁸Institute of Gerontology, The University of Georgia, Athens, GA

⁹Department of Pathology, Emory University, Atlanta, GA

¹⁰Brigham and Women's Hospital and Harvard Medical School, Boston, MA

¹¹Department of Psychology, Wayne State University, Detroit MI

¹²Department of Pathology, Baptist Health Care, Lexington, KY

¹³Departments of Epidemiology and Biostatistics, Temple University, Philadelphia, PA

¹⁴Department of Medicine and Tulane Center for Aging, Tulane University, New Orleans, LA

Abstract

With an emphasis on evolving concepts in the field, we evaluated neuropathologic data from very old research volunteers whose brain autopsies were performed at University of Kentucky (UK-ADC), incorporating data from the Georgia Centenarian Study (N=49 cases included), the Nun Study (N=17), and UK-ADC (N=11) cohorts. Average age of death was 102.0 years (range: 98–107) overall. Alzheimer's disease (AD) pathology was not universal (62% with “moderate” or “frequent” neuritic amyloid plaque densities) whereas frontotemporal lobar degeneration (FTLD)

*Corresponding Author: Peter T. Nelson MD PhD, Department of Pathology, Division of Neuropathology, Rm 311, Sanders-Brown Center on Aging, 800 S. Limestone Avenue, University of Kentucky, Lexington, KY 40536-0230, Tel: 859.218.3862, pnels2@email.uky.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

was absent. By contrast, some hippocampal neurofibrillary tangles (including primary age-related tauopathy [PART]) were observed in every case. Lewy body pathology was seen in 16.9% of subjects, hippocampal sclerosis of aging (HS-Aging) in 20.8%. We describe anatomical distributions of pigment-laden macrophages, expanded Virchow-Robin spaces, and arteriolosclerosis among Georgia Centenarians. Moderate or severe arteriolosclerosis pathology, throughout the brain, was associated with both HS-Aging pathology and an *ABCC9* gene variant. These results provide fresh insights into the complex cerebral multimorbidity, and a novel genetic risk factor, at the far end of the human aging spectrum.

Introduction

Among clinicians and researchers, there is an increasing appreciation of the heterogeneous nature of pathologies in the brains of persons who survive to extreme old age. The published literature includes multiple studies of centenarians that came to autopsy. Research subjects in those studies were characterized neuropathologically with regard to the presence and severities of Alzheimer's disease (AD), Lewy body diseases (LBD), hippocampal sclerosis of aging (HS-Aging), cerebrovascular diseases (CVD), and other neuropathologic features (Giannakopoulos, et al., 2008, Giannakopoulos, et al., 1995a, Giannakopoulos, et al., 1993, Giannakopoulos, et al., 1995b, Gold, et al., 2000, Imhof, et al., 2007, Itoh, et al., 1998, Miller, et al., 2010, Mizutani and Shimada, 1992, von Gunten, et al., 2010). In addition to prior case series, there have been excellent reviews of the findings (Hof, et al., 1996, Imhof, et al., 2007, von Gunten, et al., 2010). Both practical and theoretical challenges have been identified in terms of accurate clinical-pathological correlation in centenarians (Ding, et al., 2006a, Ding, et al., 2006b, Garcia-Sierra, et al., 2000, Gold, et al., 2000, Jellinger and Attems, 2010b, Nelson, et al., 2011b, Poon, et al., 2007, Silver, et al., 2002, Wang, et al., 1999), and most of the autopsy series that focused on centenarians have been relatively small.

In addition to what can be learned from prior studies, there are some new ideas and pathologic designations based on an evolving understanding in the field, including increased appreciation of the complexities of human brain diseases. Awareness is growing that the medical conditions among extremely old individuals may be distinct in important ways from those that affect individuals in the 70–90 year age range (Arnold, et al., 2010, Evert, et al., 2003, Nelson, et al., 2011a, Richmond, et al., 2012). There also remain some controversial issues. For example, a hypothesis has been proffered that there is a “dissociation” between pathology and clinical outcomes among the “oldest-old” (Imhof, et al., 2007, Savva, et al., 2009), but this hypothesis has also been countered (Nelson, et al., 2012). Also, the pathologic condition characterized by predominantly subcortical/hippocampal neurofibrillary tangles (NFTs) without amyloid plaques in the elderly was recently termed primary age-related tauopathy (PART) (Crary, et al., 2014). There have been arguments presented for and against the hypothesis that the pathologically-defined PART cases should be considered a distinct condition or a subset of AD (Braak and Del Tredici, 2014, Duyckaerts, et al., 2015, Jack, 2014, Jellinger, et al., 2015). A salient consideration is whether or not PART inevitably progresses to full-blown AD. It has been shown that ~20% of individuals have PART pathology by their ninth decade (the remainder some degree of

AD with amyloid plaques) (Braak, et al., 2011), so a centenarian group of comparable size with PART would seem to argue against the hypothesis that PART cases tend to progress inevitably to AD.

There also are diagnostic “border zones” that are awaiting clearer definitions, such as is the case for HS-Aging and aging-related hippocampal TDP-43-immunoreactive inclusions. These common pathologic features overlap with each other (Amador-Ortiz, et al., 2007), and both have been associated with cognitive impairment in aging (Keage, et al., 2014, Nelson, et al., 2010a). However, there is no consensus-based diagnostic rubric or nomenclature. Studies prior to 2006 were necessarily unaware that TDP-43 pathology even existed (Neumann, et al., 2006). It has been suggested that TDP-43 pathology seen in aged individuals may be a “forme fruste” (atypical, early, or otherwise diminished) manifestation of frontotemporal lobar degeneration (FTLD)-type pathogenetic changes (Dickson, 2009). Many nonagenarians (5–30% in different autopsy series) have HS-Aging with TDP-43 pathology (Kovacs, et al., 2013, Leverenz, et al., 2002, Nag, et al., 2015, Nelson, et al., 2011a, Nelson, et al., 2013, Zarow, et al., 2012). It is interesting therefore to test whether there is any evidence of disease progression in the subsequent age group – is there an appreciable subset of centenarians with full-blown FTLD-TDP?

The goals of the present study were to obtain insights into the pathologies of extreme old age, emphasizing evolving and/or controversial concepts. To address these issues in a relatively large sample of research volunteers followed to autopsy, we here report data from the combined cohorts of the Georgia Centenarian Study (GCS; (Poon, et al., 1992)), The Nun Study (Snowdon, et al., 1997, Tyas, et al., 2007), and the University of Kentucky Alzheimer’s Disease center (UK-ADC; (Schmitt, et al., 2012)). The neuropathologic assessments were all performed and analyzed at the same research center (UK-ADC). We also examined the actuarial tables from the U.S. Social Security Administration to frame the context of the study and to help convey the survival bias that relates to this group of individuals. We previously identified a single nucleotide polymorphism (SNP) which was associated with risk for HS-Aging pathology (Nelson, et al., 2014, Nelson, et al., 2015), and here we tested whether that gene variant (rs704178/rs704180 in the *ABCC9* gene) is associated with autopsy-confirmed HS-Aging and brain arteriosclerosis pathologies among individuals of extreme old age.

Methods

All protocols were performed with IRB approval from the respective institutions. Patients who came to autopsy from UK-ADC, Nun Study (Wolf, et al., 1999), and GCS (Poon, et al., 2007) cohorts were the basis for the study. Details of UK-ADC, Nun Study, and GCS recruitment have been described elsewhere (Arnold, et al., 2010, Gosche, et al., 2002, Hensley, et al., 2010, Nelson, et al., 2007, Poon, et al., 1992, Riley, et al., 2002, Schmitt, et al., 2001). Mental status testing (Schmitt, et al., 2000) employed cognitive instruments that included the Mini-Mental State Examination (MMSE; (Davey, et al., 2013, Folstein, et al., 1975)).

Pathological assessments were performed at the University of Kentucky on all the cases, and the methodology has been described in detail (Davis, et al., 1999, Nelson, et al., 2009a, Nelson, et al., 2007, Riley, et al., 2002, Wolf, et al., 1999). Braak NFT staging and CERAD quantification of neuritic amyloid plaques were as described previously (Braak and Braak, 1991, Mirra, 1997). Lewy body pathologies were evaluated according to the consensus-based recommendations (McKeith, et al., 2004, McKeith, et al., 2000). The neuropathological criterion for HS-Aging was neuron loss and gliosis in the hippocampal formation, not readily ascribable to another pathology such as neurofibrillary tangles or localizable infarction (Montine, et al., 2012a, Nelson, et al., 2013). Aberrant TDP-43 immunohistochemistry was performed as described previously and refers to staining that is cytoplasmic, neuritic, or tangle-like (Nelson, et al., 2011b). For *ABCC9* SNP analyses, DNA was obtained from fresh (frozen) tissue and the SNP characterized as previously described (Nelson, et al., 2014) using Life Technologies' TaqMan-based SNP assays. Otherwise the results relate to findings on available hematoxylin and eosin (H&E) stained slides.

Semi-quantitative assessment of the vascular pathologies was performed on all available H&E stained slides for each of the GCS cohort's cases (22 different brain regions). These data were collected blinded to all clinical information and previous pathology diagnoses and were scored according to semiquantitative scoring methods: Virchow Robin space alterations were graded based on two parameters: the severity around a given vessel and the degree of involvement of vessels throughout the section. The severity was graded on a four point scale, ranging from 0 to 3+. The degree of involvement was graded in quartiles (0, 1–25%, 26–50%, 51–75%, 76–100%).

The presence of perivascular pigment-laden macrophages was also documented using H&E stained sections. The entire slide in each section was examined (both gray and white matter) for the presence of perivascular macrophages. The number of vessels involved in both gray and white matter were combined to generate a single result. Up to four vessels were recorded individually; if the number of vessels involved was greater than four, the data were collapsed into a “ 5” category.

For statistical analyses of arteriolosclerosis pathology in the GCS data, data were imported into SAS/STAT 9.3®. All arteriolosclerosis ratings in 22 brain regions were coded according to a 0–3 severity scale. Less than 4% of slides were missing in terms of H&E evaluation of arteriolosclerosis severity (26 cases had complete data, 10 cases were missing one region, 10 cases were missing two regions, and three cases were missing three regions). For the comparisons we wished to perform (see below), the main research questions related to the frequency of moderate-to-severe arteriolosclerosis in the sampled brain regions. The arteriolosclerosis ratings were recoded into dummy indicators for presence of at least moderate arteriolosclerosis versus absent or mild severity. Indicators for each case were summed to produce a count of brain regions with at least moderate arteriolosclerosis. The number of affected regions was scaled by the number of regions with nonmissing data. In separate models, we used Poisson regression to estimate the effect of TDP-43, HS-Aging, and AD pathologies, and *ABCC9* genotype, on mean number of regions with at least moderate arteriosclerosis. In each model, pathology or genotype group was the only predictor.

Results

The research cohort comprised a sample of very old individuals (n=77 total) accrued by combining data from the GCS (n=49), Nun Study (n=17), and UK-ADC (n=11) autopsy cohorts; see Table 1. Average overall age at death was 102.0 years (range 98–107; 8 cases were 98 or 99 years old at death). Many of the subjects were cognitively impaired before dying (average final MMSE score was 15.5 out of 30, range 0–30). One individual had final MMSE score of 30, five individuals had final MMSE scores of 28, each of these six brains had Braak NFT stages $\leq$ III. The individual with final MMSE score of 30 was a woman that lived independently and had Braak NFT stage of II but no amyloid plaques. None of the research volunteers lacked any NFTs. For those with Braak NFT stages I–II, the average final MMSE score was 20.0 (stdev 7.4). By contrast, among those with Braak NFT stages V or VI (n=22), the average final MMSE score was 9.1 (stdev 8.6). The entire sample was skewed toward females (69/77 cases). See supplemental material for a table that contains detailed clinical and pathologic description on each research subject.

To provide contextual information on the human aging spectrum in relation to the age of the individuals in the current study, we accessed actuarial data from the Social Security Administration (Fig. 1). These provide information from an authoritative source about the proportion of individuals expected to be alive from 100,000 births, and the “survival curves” include both past data and future predictions. As expected, females have longer lifespan than males. Using data referent to two dates, 1950 and 2020 projections, it is clear that a shift has been seen over time, favoring longer lifespan for both genders. That shift is more pronounced than the gender effect. However, even after that shift, <1% of individuals of both genders would be expected by the year 2020 to live beyond their 102nd birthday, which indicates that the current study sample reflects the very end of the human aging spectrum, at least for the near future.

Among the research volunteers included in the present study, AD-type changes – neurofibrillary tangles (NFTs) and amyloid plaques – were frequently seen. The pathologic stages defined by Braak and Braak (NFT Stages), and CERAD neuritic plaque densities are shown in Table 2. For this table, five cases were not given a Braak stage due to extensive HS-Aging pathology. Further analyses revealed that there was some variation between the different research cohorts, but, in each, there was a substantial minority of individuals with no neuritic amyloid plaques (NPs; CERAD “none”; Fig 2). The variation between cohorts may reflect the small sample sizes in each cohort. Moreover, in the combined group, 37% of cases had either “None” or “Sparse” NPs. Approximately 21% in the combined group had NFTs without NPs (i.e., PART), and there were no cases with amyloid plaques but no NFTs because all cases had some NFTs (Fig. 3).

Non-AD pathologies were predominantly DLB, HS-Aging/TDP-43, and CVD. The DLB pathology was either “limbic-type” (9/77, 12%) or “neocortical type” (4/77, 5%); Table 3. Hippocampal sclerosis pathology was seen on H&E stain in 20.8% (16/77) of cases, with 12/15 (80%) cases tested immunohistochemically being positive for TDP-43 pathology in the hippocampal formation (Table 4). There were nine cases (12% of the cohort) with hippocampal TDP-43 pathology but no HS seen by H&E stain, with the caveat that TDP-43

could not be assessed in 9 of the cases. The majority of individuals in the study had more than one subtype of comorbid pathology (Fig. 4). In this assessment, we operationalized different neuropathologic subtypes as “positive” if the case showed: Braak NFT stage>II (thus, either “intermediate severity” AD neuropathologic changes, or PART), Cerebral amyloid angiopathy (moderate or severe), arteriolosclerosis (moderate or severe), HS-Aging or TDP-43 pathology, Lewy body disease, or large infarcts.

For further studies, related to cerebrovascular pathologies (Figs. 5–6, Tables 5–6), we concentrated on a subset of the aged cohort, which had very uniform pathological workup: the GCS study participants (n=49), whose recruitment is describe elsewhere (Arnold, et al., 2010, Shaw, et al., 2012). For these research participants, an identical panel of 22 different brain blocks was assessed evaluating both sides of the brain, followed by semiquantitative counts of three pathologic features that were graded (blind to all other information, on a zero-to-three scale) by a single neuropathologist (JN): arteriolosclerosis, pigment-laden macrophages, and expanded Virchow-Robin spaces. The grading scheme is depicted in Fig. 5. The overall results of the cerebrovascular pathologic assessments in the GCS cohort are presented in Fig. 6. In this figure, the average degree of pathology scored in each of the brain regions is presented.

In addition to providing an opportunity to describe the brain distribution of arteriolosclerosis, pigment-laden macrophages, and expanded Virchow-Robin spaces, the scored data provided an opportunity to test specific hypotheses: first, is brain arteriolosclerosis outside of the hippocampus associated with HS-Aging pathology in the GCS, as previously shown in other cohorts (Neltner, et al., 2014)? And secondly, does the *ABCC9* risk SNP previously linked to HS-Aging (Nelson, et al., 2014, Nelson, et al., 2015) also show association with brain arteriolosclerosis in this cohort? The results of these tests are shown in Tables 5 and 6. The GCS data provide support for the above hypotheses. In comparing arteriolosclerosis ratings between cases with, versus without HS-Aging pathology, estimated mean number of regions with arteriolosclerosis for HS-Aging+ cases = 8.67 (95% CI 6.60, 11.37), for HS-Aging– cases = 5.26 (4.61, 5.99); $p = 0.0011$. For cases with TDP-43 pathology, the estimated mean number of regions = 7.36 (95% CI 6.07, 8.92), for TDP-43– cases = 5.38 (4.60, 6.29); $p = 0.0137$. By contrast, for cases with advanced AD pathology, mean number of arteriolosclerosis regions = 6.0 (95% CI 4.88, 7.38), versus lacking that pathology = 5.53 (4.79, 6.38); $p = 0.52$. Extending the same analyses to *ABCC9* genotypes, comparing homozygous G_G versus (G_C or C_C), the estimated mean number of regions with arteriolosclerosis for G_G = 7.10 (95% CI 5.63, 8.96), for G_C & C_C = 5.20 (4.50, 6.01); $p = 0.026$. Leaving out the HS-Aging cases, but performing the same analysis otherwise, the estimated mean number of regions for G_G = 7.13 (95% CI 5.50, 9.24), for G_C & C_C = 4.81 (4.11, 5.64); $p = 0.0114$. Finally, comparing just the homozygous cases (G_G versus C_C), the estimated mean number of regions for G_G = 7.10 (95% CI 5.63, 8.96), for C_C = 4.11 (2.98, 5.67); $p = 0.007$.

Discussion

We describe neuropathologic observations in a large sample of research subjects relative to prior studies in this age range. These data underscore both the complexity of brain

pathologies in advanced old age, and the evolving nature of the field studying them. Among these extremely old research subjects, AD-type pathology was not universal, whereas some degree of hippocampal NFTs (including PART cases) was seen in all cases. Both LBD and HS-Aging pathologies were common, but by no means universal, among centenarians in this sample. By contrast, small vessel CVD was very prevalent. Brains with widespread moderate or severe arteriolosclerosis pathology were relatively likely to also harbor HS-Aging pathology (versus those that lacked HS-Aging pathology) and to be carriers of the rs704180 G_G genotype (in comparison to G_C and C_C carriers). To help frame the significance of these results, we presented data from the United States Social Security Administration's actuarial tables, indicating that fewer than 1% of Americans live to the age of this study cohort (average 102.0 years at death), so these findings relate to the oldest portion of human aging.

There are some limitations to our study. The individuals derived from three separate cohorts (UK-ADC cohort is associated with a memory disorder clinic, the other two are population-based samples), rather than from a homogenous study cohort. Further, all were evaluated over a course of many years, and in that time some of the methodologies of neuropathologic evaluations have changed. Specifically, contrary to recent consensus recommendations for AD neuropathologic diagnosis (Montine, et al., 2012b), there was no Thal staging (Thal, et al., 2006) of A β immunohistochemistry available for most of the cases, so this was left out of the study and represents a study limitation. It would have been challenging to perform A β immunohistochemistry on all the cases retrospectively. Further, our evaluation of pigment-laden macrophages leaves open the question of whether all of these cells have hemosiderin, or some having other pigment (perhaps including lipofuscin). We have not found a perfect correlation between pigmented macrophages and the cells that are stained with Prussian Blue on histology (data not shown). We also left unaddressed some of the other emerging concepts in neuropathology such as age-related astrocytic tauopathy (Ferrer, et al., 2014). Although these considerations are important, there is also a positive tradeoff since the methodology was relatively standardized, all being performed at the same institution. Even with that strength, the issues related to plaque and tangle quantification are complex (both technically and theoretically) and pathologic factors may not align perfectly with rigid classification schemes.

More theoretically, an assumption is that centenarians provide insights into mechanisms related to "aging", but the concept of aging is not universally defined in a biologic sense, beyond chronologic persistence. Any study of centenarians involves a strong survival bias so that many of the individuals that have been removed from consideration (by death, or by otherwise not participating as a research volunteer), may have manifested "aging" in different ways. A specific example of such a potential source of bias is that women, who live longer than men on average (Figure 1), also are less likely to develop neocortical LBD (Nelson, et al., 2010b), which may help explain the relative lack of Lewy body pathology (no Parkinson's disease patients) in this sample which is almost 90% female. Additional factors may cause or exacerbate parkinsonism in older individuals (Buchman, et al., 2012, Hack, et al., 2012). Finally, there is a possibility that future efforts will enable people to live longer (Christensen, et al., 2009), perhaps even much longer, so that the age of 102 years may not represent the true "final stage" of human longevity. Whereas this theoretically may

be true, we note that, as of November 2014, in the entire world, there were only estimated to be 300–450 individuals over the age of 110 (only 79 validated at that time; see (Robert and Fulop, 2014) and <http://www.grg.org/Adams/E.HTM>), out of >7,000,000,000 alive, so the potential for widespread lifespan attainment much beyond that is theoretical indeed.

Despite the potential challenges, there are important insights that can be gained by studying centenarians. Prior studies have yielded excellent information in this area, although some controversies and questions remain. One clear implication of the current study is that AD (the malignant plaque and tangle disease) is not inevitable in advanced old age although it is the most frequent and highly impactful neurodegenerative condition in this group. This result agrees with a prior study in a large extremely high-quality autopsy sample that found ~20% of centenarian cases lack A β by immunohistochemistry (Thal A β stage 0) (Braak, et al., 2011). It is intriguing that the *APOE* ϵ 4 genotype in the present sample (only 11 individuals out of 76 tested were ϵ 4 allele carriers) is lower than most populations (Singh, et al., 2006), possibly indicating a survival effect as suggested previously ((Jicha, et al., 2008); but see (Corrada, et al., 2013)). Clearly, some degree of hippocampal NFTs develop even in the absence of amyloid plaques, albeit an attenuated distribution (Crary, et al., 2014, Jellinger, et al., 2015, Nelson, et al., 2009b). Relative to slightly younger cohorts, the overall proportion of AD and PART cases seems to be stable in advanced old age, supporting the hypothesis that PART pathology is not necessarily destined to progress to full-blown AD. By contrast, in this cohort there were no “plaque-only” cases because all brains had some hippocampal NFTs.

Our study also provides further support for the prevalence of non-AD pathologies in advanced old age (Attems, et al., 2014, Brenowitz, et al., 2015, Corrada, et al., 2012, Dickson, 2009, Erten-Lyons, et al., 2013, Jellinger and Attems, 2010a, Jellinger and Attems, 2010b, Jicha, et al., 2012, Kawas, et al., 2015, Kovacs, et al., 2013, Magaki, et al., 2014, Serrano-Pozo, et al., 2013, Sonnen, et al., 2011, Toledo, et al., 2013). Here we found extensive co-occurrence of CVD, AD, HS-Aging, and alpha-synucleinopathy, and as expected, most brains contained more than one subtype of pathology. These common cerebral multimorbidities help to explain the apparent “dissociation” between clinical and pathological parameters in correlation analyses that only focus on a single subtype of pathology (Nelson, et al., 2012, Scheff, et al., 2014). Any determination of specific relationships between cognition and pathology in extreme old age requires either culling out for analysis the rare cases of “pure” (single-pathology) examples of that pathologic subtype, or, formulating multiple variable approaches that require large cohorts, carefully-applied statistical models, and numerous – preferably non-ordinal – quantitative pathologic parameters. The current study, assessing the numerous pathologies of the “oldest-old” among relatively few research subjects, lacked adequate statistical power to accomplish robust clinical-pathologic correlation.

In terms of pathologies with established impact on cognition, there is an emerging appreciation of the strong impact of HS-Aging and TDP-43 pathologies (Nelson, et al., 2010a, Nelson, et al., 2013). As shown previously (Amador-Ortiz, et al., 2007, Nelson, et al., 2011b), most cases with HS-Aging pathology also showed TDP-43 pathology (79% of HS-Aging cases in the current cohort were TDP-43+). While we showed previously that HS-

Aging pathology increases in advanced old age (as opposed to the prevalence of AD, CAA, or DLB pathologies, which level off, or even decreases) (Brenowitz, et al., 2014, Brenowitz, et al., 2015, Nelson, et al., 2011a, Nelson, et al., 2011b), the present study indicates that HS-Aging pathology, and TDP-43 pathology which frequently coexists with HS-Aging, are by no means universal among centenarians. If hippocampal TDP-43 were an early manifestation of a disease process that would ultimately lead to full-blown FTLTDP, then there should be an appreciable subset of very old people with an overall pathologic status that resembles FTLTDP with extremely widespread TDP-43 pathology. This does not appear to be the case. However, there may well be pathogenetic overlap between aging-related hippocampal TDP-43 pathology and FTLTDP as underscored by genetic data presented in other studies (Aoki, et al., 2015, Fenoglio, et al., 2009, Murray, et al., 2014, Nelson, et al., 2015, Rademakers, et al., 2008, Rutherford, et al., 2012).

Where HS-Aging and FTLTDP may differ relates to the potential impact of arteriolosclerosis on the brains of individuals in advanced old age. We previously showed that arteriolosclerosis outside of the hippocampus is increased in persons with comorbid HS-Aging pathology, versus controls (Neltner, et al., 2014). Further, an *ABCC9* SNP is associated with risk for HS-Aging pathology (Nelson, et al., 2014, Nelson, et al., 2015), and the current study indicates that the same SNP is also a risk factor for brain arteriolosclerosis (additional work is being performed in our lab to test this hypothesis). Acute vascular injury does not induce HS-Aging or TDP-43 pathologies (Lee, et al., 2008), but chronic vascular injury may do so. As an analogous concept, acute brain trauma does not result in TDP-43 pathology (Johnson, et al., 2011), but chronic brain trauma often does (King, et al., 2010, Saing, et al., 2011, Smith, et al., 2013). Our data indicate that a disproportionate subset of cases with (presumably chronic) brain arteriolosclerosis develop HS-Aging pathology (Neltner, et al., 2014), both linked to *ABCC9* gene variants. Importantly, the cases included in the current study were not also used in the Neltner et al (2014) paper, so this is a validation of the prior report indicating that HS-Aging is just one manifestation of a “whole brain” disease with arteriolosclerosis widespread outside of the hippocampus. We also note that the association between HS-Aging and brain arteriolosclerosis pathologies in advanced old age aligns well with results of prior studies (Chui, et al., 2006, Dickson, et al., 1994, Jellinger, 2007, Pantoni, et al., 1996, Snowden, et al., 1997, White, 2009). Collectively, these findings indicate that HS-Aging pathology may be the manifestation of a disease with attributes that are seen in both cerebrovascular and neurodegenerative conditions.

Finally, the present study addressed some cerebrovascular neuropathologic features that are not standardized in terms of universally-applied neuropathological guidelines: pigment-laden macrophages, expanded Virchow-Robin spaces, and arteriolosclerosis. For the purposes of the current study, these results are purely descriptive. The data are presented to help convey how the various CVD subtypes are distributed in the brains of individuals in this cohort. We were struck that some areas of the brain that are not necessarily frequently associated with CVD (e.g., insula cortex) showed a very high burden of small vessel pathology in this sample. Further, it would appear that microscopic extravasation of blood is common among centenarians since many cerebral cortical areas showed pigmented macrophages near blood vessels. These changes may help explain the prior discovered

alterations in small blood vessel profiles (Imhof, et al., 2007). Future work is required to better understand the cerebrovascular pathologies in the “oldest-old”.

We conclude that the study of extremely old individuals’ brains provide scientific insights into brain diseases and aging in general. FTLT seems to mainly manifest in younger individuals. We have not seen a case that lacks some degree of hippocampal NFTs unless the hippocampus was obliterated by sclerotic changes. Yet we confirm prior studies (Garcia-Sierra, et al., 2000, Gold, et al., 2000, Imhof, et al., 2007, Itoh, et al., 1998, Mizutani and Shimada, 1992) that indicate variability in the severity of this pathology in centenarians. Because none of these other neurodegenerative disease pathologies (AD, FTLT, HS-Aging, or LBD) is universal among centenarians, our data can be interpreted optimistically to support the hypothesis that the “neurodegenerative diseases” can be addressed by future therapeutic strategies to counteract the genetic and environmental factors (many probably as-yet unknown) that lead to these pathologies. However, there are aspects of aging that are more likely to be an inevitable manifestation of the human genetic blueprint. The aged brain, as with other organs, seems to be often afflicted with small blood vessel degenerative changes. Presumably there are some risk factors (diabetes, hypertension) that can exacerbate blood vessel pathology among individuals of all ages. However, it remains to be determined whether cerebral small blood vessel changes in extreme old age can be therapeutically eliminated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are deeply grateful to all of the study volunteers. We thank Sonya Anderson and Ela Patel for technical support, Greg Cooper, MD, Nancy Stiles, MD and Allison Caban-Holt, PhD for the clinical evaluations. This study was supported by NIH grants R01 NS061933, R01 AG19241, P01 AG17553, P30 AG028383, and U01 AG016976.

Abbreviations

CVD	cerebrovascular disease
FTLT	frontotemporal lobar degeneration
HS-Aging	hippocampal sclerosis of aging
LBD	Lewy body disease
NFTs	neurofibrillary tangles
PART	primary age-related tauopathy
TDP-43	TAR-DNA binding protein-43

Bibliography

Amador-Ortiz C, Lin WL, Ahmed Z, Personett D, Davies P, Duara R, Graff-Radford NR, Hutton ML, Dickson DW. TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer’s disease. *Annals of neurology*. 2007; 61(5):435–45. [PubMed: 17469117]

- Aoki N, Murray ME, Ogaki K, Fujioka S, Rutherford NJ, Rademakers R, Ross OA, Dickson DW. Hippocampal sclerosis in Lewy body disease is a TDP-43 proteinopathy similar to FTLTD-TDP Type A. *Acta Neuropathol.* 2015; 129(1):53–64. [PubMed: 25367383]
- Arnold J, Dai J, Nahapetyan L, Arte A, Johnson MA, Hausman D, Rodgers WL, Hensley R, Martin P, Macdonald M, Davey A, Siegler IC, Jazwinski SM, Poon LW. Predicting successful aging in a population-based sample of georgia centenarians. *Curr Gerontol Geriatr Res.* 2010
- Attems J, Neltner JH, Nelson PT. Quantitative neuropathological assessment to investigate cerebral multi-morbidity. *Alzheimer's research & therapy.* 2014; 6(9):85.
- Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol.* 1991; 82(4):239–59. [PubMed: 1759558]
- Braak H, Del Tredici K. Are cases with tau pathology occurring in the absence of Abeta deposits part of the AD-related pathological process? *Acta Neuropathol.* 2014; 128(6):767–72. [PubMed: 25359108]
- Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *Journal of neuropathology and experimental neurology.* 2011; 70(11):960–9. [PubMed: 22002422]
- Brenowitz WD, Monsell SE, Schmitt FA, Kukull WA, Nelson PT. Hippocampal sclerosis of aging is a key Alzheimer's disease mimic: clinical-pathologic correlations and comparisons with both alzheimer's disease and non-tauopathic frontotemporal lobar degeneration. *J Alzheimers Dis.* 2014; 39(3):691–702. [PubMed: 24270205]
- Brenowitz WD, Nelson PT, Besser LM, Heller KB, Kukull WA. Cerebral amyloid angiopathy and its co-occurrence with Alzheimer's disease and other cerebrovascular neuropathologic changes. *Neurobiology of aging.* 2015
- Buchman AS, Shulman JM, Nag S, Leurgans SE, Arnold SE, Morris MC, Schneider JA, Bennett DA. Nigral pathology and parkinsonian signs in elders without Parkinson disease. *Annals of neurology.* 2012; 71(2):258–66. [PubMed: 22367997]
- Christensen K, Doblhammer G, Rau R, Vaupel JW. Ageing populations: the challenges ahead. *Lancet.* 2009; 374(9696):1196–208. [PubMed: 19801098]
- Chui HC, Zarow C, Mack WJ, Ellis WG, Zheng L, Jagust WJ, Mungas D, Reed BR, Kramer JH, Decarli CC, Weiner MW, Vinters HV. Cognitive impact of subcortical vascular and Alzheimer's disease pathology. *Annals of neurology.* 2006; 60(6):677–87. [PubMed: 17192928]
- Corrada MM, Berlau DJ, Kawas CH. A population-based clinicopathological study in the oldest-old: the 90+ study. *Curr Alzheimer Res.* 2012; 9(6):709–17. [PubMed: 22471863]
- Corrada MM, Paganini-Hill A, Berlau DJ, Kawas CH. Apolipoprotein E genotype, dementia, and mortality in the oldest old: the 90+ Study. *Alzheimers Dement.* 2013; 9(1):12–8. [PubMed: 23123227]
- Crary JF, Trojanowski JQ, Schneider JA, Abisambra JF, Abner EL, Alafuzoff I, Arnold SE, Attems J, Beach TG, Bigio EH, Cairns NJ, Dickson DW, Gearing M, Grinberg LT, Hof PR, Hyman BT, Jellinger K, Jicha GA, Kovacs GG, Knopman DS, Kofler J, Kukull WA, Mackenzie IR, Masliah E, McKee A, Montine TJ, Murray ME, Neltner JH, Santa-Maria I, Seeley WW, Serrano-Pozo A, Shelanski ML, Stein T, Takao M, Thal DR, Toledo JB, Troncoso JC, Vonsattel JP, White CL 3rd, Wisniewski T, Woltjer RL, Yamada M, Nelson PT. Primary age-related tauopathy (PART): a common pathology associated with human aging. *Acta Neuropathol.* 2014; 128(6):755–66. [PubMed: 25348064]
- Davey A, Dai T, Woodard JL, Miller LS, Gondo Y, Johnson MA, Hausman DB, Martin P, Green RC, Allen RH, Stabler SP, Poon LW, Georgia C. Profiles of cognitive functioning in a population-based sample of centenarians using factor mixture analysis. *Exp Aging Res.* 2013; 39(2):125–44. [PubMed: 23421635]
- Davis DG, Schmitt FA, Wekstein DR, Markesbery WR. Alzheimer neuropathologic alterations in aged cognitively normal subjects. *Journal of neuropathology and experimental neurology.* 1999; 58(4): 376–88. [PubMed: 10218633]
- Dickson DW. Neuropathology of non-Alzheimer degenerative disorders. *International journal of clinical and experimental pathology.* 2009; 3(1):1–23. [PubMed: 19918325]

- Dickson DW, Davies P, Bevona C, Van Hoeven KH, Factor SM, Grober E, Aronson MK, Crystal HA. Hippocampal sclerosis: a common pathological feature of dementia in very old (> or = 80 years of age) humans. *Acta Neuropathol.* 1994; 88(3):212–21. [PubMed: 7810292]
- Ding ZT, Wang Y, Jiang YP, Hashizume Y, Yoshida M, Mimuro M, Inagaki T, Iwase T. Characteristics of alpha-synucleinopathy in centenarians. *Acta Neuropathol.* 2006a; 111(5):450–8. [PubMed: 16520971]
- Ding ZT, Wang Y, Jiang YP, Yoshida M, Mimuro M, Inagaki T, Iwase T, Hashizume Y. Argyrophilic grain disease: frequency and neuropathology in centenarians. *Acta Neuropathol.* 2006b; 111(4): 320–8. [PubMed: 16525805]
- Duyckaerts C, Braak H, Brion JP, Buee L, Del Tredici K, Goedert M, Halliday G, Neumann M, Spillantini MG, Tolnay M, Uchihara T. PART is part of Alzheimer disease. *Acta Neuropathol.* 2015; 129(5):749–56. [PubMed: 25628035]
- Erten-Lyons D, Dodge HH, Woltjer R, Silbert LC, Howieson DB, Kramer P, Kaye JA. Neuropathologic basis of age-associated brain atrophy. *JAMA neurology.* 2013; 70(5):616–22. [PubMed: 23552688]
- Evert J, Lawler E, Bogan H, Perls T. Morbidity profiles of centenarians: survivors, delayers, and escapers. *The journals of gerontology Series A, Biological sciences and medical sciences.* 2003; 58(3):232–7.
- Fenoglio C, Galimberti D, Cortini F, Kauwe JS, Cruchaga C, Venturelli E, Villa C, Serpente M, Scalabrini D, Mayo K, Piccio LM, Clerici F, Albani D, Mariani C, Forloni G, Bresolin N, Goate AM, Scarpini E. Rs5848 variant influences GRN mRNA levels in brain and peripheral mononuclear cells in patients with Alzheimer's disease. *J Alzheimers Dis.* 2009; 18(3):603–12. [PubMed: 19625741]
- Ferrer I, Lopez-Gonzalez I, Carmona M, Arregui L, Dalfo E, Torrejon-Escribano B, Diehl R, Kovacs GG. Glial and neuronal tau pathology in tauopathies: characterization of disease-specific phenotypes and tau pathology progression. *Journal of neuropathology and experimental neurology.* 2014; 73(1):81–97. [PubMed: 24335532]
- Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* 1975; 12(3):189–98. [PubMed: 1202204]
- Garcia-Sierra F, Hauw JJ, Duyckaerts C, Wischik CM, Luna-Munoz J, Mena R. The extent of neurofibrillary pathology in perforant pathway neurons is the key determinant of dementia in the very old. *Acta Neuropathol.* 2000; 100(1):29–35. [PubMed: 10912917]
- Giannakopoulos P, Bouras C, Hof PR. Clinicopathologic correlates in the oldest-old: Commentary on "No disease in the brain of a 115-year-old woman". *Neurobiology of aging.* 2008; 29(8):1137–9. [PubMed: 18534719]
- Giannakopoulos P, Hof PR, Giannakopoulos AS, Herrmann FR, Michel JP, Bouras C. Regional distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of very old patients. *Archives of neurology.* 1995a; 52(12):1150–9. [PubMed: 7492288]
- Giannakopoulos P, Hof PR, Surini M, Michel JP, Bouras C. Quantitative immunohistochemical analysis of the distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of nonagenarians and centenarians. *Acta neuropathologica.* 1993; 85(6):602–10. [PubMed: 8337938]
- Giannakopoulos P, Hof PR, Vallet PG, Giannakopoulos AS, Charnay Y, Bouras C. Quantitative analysis of neuropathologic changes in the cerebral cortex of centenarians. *Prog Neuropsychopharmacol Biol Psychiatry.* 1995b; 19(4):577–92. [PubMed: 8588057]
- Gold G, Bouras C, Kovari E, Canuto A, Glaria BG, Malky A, Hof PR, Michel JP, Giannakopoulos P. Clinical validity of Braak neuropathological staging in the oldest-old. *Acta neuropathologica.* 2000; 99(5):579–82. discussion 83–4. [PubMed: 10805104]
- Gosche KM, Mortimer JA, Smith CD, Markesbery WR, Snowdon DA. Hippocampal volume as an index of Alzheimer neuropathology: findings from the Nun Study. *Neurology.* 2002; 58(10):1476–82. [PubMed: 12034782]
- Hack N, Jicha GA, Abell A, Dean D, Vitek JL, Berger JR. Substantia nigra depigmentation and exposure to encephalitis lethargica. *Annals of neurology.* 2012; 72(6):912–7. [PubMed: 23280841]

- Hensley B, Martin P, MacDonald M, Poon L, Jazwinski SM, Green RC, Gearing M, Markesbery WR, Woodard JL, Johnson MA, Tenover JS, Siegler IC, Rodgers WL, Hausman DB, Rott C, Davey A, Arnold J. Family history and adaptation among centenarians and octogenarians. *Gerontology*. 2010; 56(1):83–7. [PubMed: 20110721]
- Hof PR, Glannakopoulos P, Bouras C. The neuropathological changes associated with normal brain aging. *Histol Histopathol*. 1996; 11(4):1075–88. [PubMed: 8930649]
- Imhof A, Kovari E, von Gunten A, Gold G, Rivara CB, Herrmann FR, Hof PR, Bouras C, Giannakopoulos P. Morphological substrates of cognitive decline in nonagenarians and centenarians: A new paradigm? *J Neurol Sci*. 2007
- Itoh Y, Yamada M, Suematsu N, Matsushita M, Otomo E. An immunohistochemical study of centenarian brains: a comparison. *J Neurol Sci*. 1998; 157(1):73–81. [PubMed: 9600680]
- Jack CR Jr. PART and SNAP. *Acta Neuropathol*. 2014; 128(6):773–6. [PubMed: 25380757]
- Jellinger KA. The enigma of vascular cognitive disorder and vascular dementia. *Acta Neuropathol*. 2007; 113(4):349–88. [PubMed: 17285295]
- Jellinger KA, Alafuzoff I, Attems J, Beach TG, Cairns NJ, Crary JF, Dickson DW, Hof PR, Hyman BT, Jack CR Jr, Jicha GA, Knopman DS, Kovacs GG, Mackenzie IR, Masliah E, Montine TJ, Nelson PT, Schmitt F, Schneider JA, Serrano-Pozo A, Thal DR, Toledo JB, Trojanowski JQ, Troncoso JC, Vonsattel JP, Wisniewski T. PART, a distinct tauopathy, different from classical sporadic Alzheimer disease. *Acta Neuropathol*. 2015; 129(5):757–62. [PubMed: 25778618]
- Jellinger KA, Attems J. Prevalence and pathology of vascular dementia in the oldest-old. *J Alzheimers Dis*. 2010a; 21(4):1283–93. [PubMed: 21504129]
- Jellinger KA, Attems J. Prevalence of dementia disorders in the oldest-old: an autopsy study. *Acta Neuropathol*. 2010b; 119(4):421–33. [PubMed: 20204386]
- Jicha GA, Abner EL, Schmitt FA, Kryscio RJ, Riley KP, Cooper GE, Stiles N, Mendiondo MS, Smith CD, Van Eldik LJ, Nelson PT. Preclinical AD Workgroup staging: pathological correlates and potential challenges. *Neurobiology of aging*. 2012; 33(3):622 e1–e16. [PubMed: 21507528]
- Jicha GA, Parisi JE, Dickson DW, Cha RH, Johnson KA, Smith GE, Boeve BF, Petersen RC, Knopman DS. Age and apoE associations with complex pathologic features in Alzheimer's disease. *J Neurol Sci*. 2008; 273(1–2):34–9. [PubMed: 18653200]
- Johnson VE, Stewart W, Trojanowski JQ, Smith DH. Acute and chronically increased immunoreactivity to phosphorylation-independent but not pathological TDP-43 after a single traumatic brain injury in humans. *Acta Neuropathol*. 2011; 122(6):715–26. [PubMed: 22101322]
- Kawas CH, Kim RC, Sonnen JA, Bullain SS, Trieu T, Corrada MM. Multiple pathologies are common and related to dementia in the oldest-old: The 90+ Study. *Neurology*. 2015; 85(6):535–42. [PubMed: 26180144]
- Keage HA, Hunter S, Matthews FE, Ince PG, Hodges J, Hokkanen S, Highley JR, Denning T, Brayne C. TDP-43 in the Population: Prevalence and Associations with Dementia and Age. *J Alzheimers Dis*. 2014
- King A, Sweeney F, Bodi I, Troakes C, Maekawa S, Al-Sarraj S. Abnormal TDP-43 expression is identified in the neocortex in cases of dementia pugilistica, but is mainly confined to the limbic system when identified in high and moderate stages of Alzheimer's disease. *Neuropathology*. 2010; 30(4):408–19. [PubMed: 20102526]
- Kovacs GG, Milenkovic I, Wohrer A, Hoftberger R, Gelpi E, Haberler C, Honigschnabl S, Reiner-Concin A, Heinzl H, Jungwirth S, Krampla W, Fischer P, Budka H. Non-Alzheimer neurodegenerative pathologies and their combinations are more frequent than commonly believed in the elderly brain: a community-based autopsy series. *Acta Neuropathol*. 2013; 126(3):365–84. [PubMed: 23900711]
- Lee EB, Lee VM, Trojanowski JQ, Neumann M. TDP-43 immunoreactivity in anoxic, ischemic and neoplastic lesions of the central nervous system. *Acta Neuropathol*. 2008; 115(3):305–11. [PubMed: 18087705]
- Leverenz JB, Agustin CM, Tsuang D, Peskind ER, Edland SD, Nochlin D, DiGiacomo L, Bowen JD, McCormick WC, Teri L, Raskind MA, Kukull WA, Larson EB. Clinical and neuropathological characteristics of hippocampal sclerosis: a community-based study. *Archives of neurology*. 2002; 59(7):1099–106. [PubMed: 12117357]

- Magaki S, Yong WH, Khanlou N, Tung S, Vinters HV. Comorbidity in dementia: update of an ongoing autopsy study. *Journal of the American Geriatrics Society*. 2014; 62(9):1722–8. [PubMed: 25039832]
- McKeith I, Mintzer J, Aarsland D, Burn D, Chiu H, Cohen-Mansfield J, Dickson D, Dubois B, Duda JE, Feldman H, Gauthier S, Halliday G, Lawlor B, Lippa C, Lopez OL, Carlos Machado J, O'Brien J, Playfer J, Reid W. Dementia with Lewy bodies. *Lancet Neurol*. 2004; 3(1):19–28. [PubMed: 14693108]
- McKeith IG, Ballard CG, Perry RH, Ince PG, O'Brien JT, Neill D, Lowery K, Jaros E, Barber R, Thompson P, Swann A, Fairbairn AF, Perry EK. Prospective validation of consensus criteria for the diagnosis of dementia with Lewy bodies. *Neurology*. 2000; 54(5):1050–8. [PubMed: 10720273]
- Miller LS, Mitchell MB, Woodard JL, Davey A, Martin P, Poon LW, Jazwinski SM, Green RC, Gearing M, Markesbery WR, Johnson MA, Tenover JS, Rodgers WL, Hausman DB, Arnold J, Siegler IC. Cognitive performance in centenarians and the oldest old: norms from the Georgia Centenarian Study. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn*. 2010; 17(5):575–90. [PubMed: 20521181]
- Mirra SS. The CERAD neuropathology protocol and consensus recommendations for the postmortem diagnosis of Alzheimer's disease: a commentary. *Neurobiology of aging*. 1997; 18(4 Suppl):S91–4. [PubMed: 9330994]
- Mizutani T, Shimada H. Neuropathological background of twenty-seven centenarian brains. *J Neurol Sci*. 1992; 108(2):168–77. [PubMed: 1517748]
- Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, Duyckaerts C, Frosch MP, Masliah E, Mirra SS, Nelson PT, Schneider JA, Thal DR, Trojanowski JQ, Vinters HV, Hyman BT. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol*. 2012a; 123(1):1–11. [PubMed: 22101365]
- Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, Duyckaerts C, Frosch MP, Masliah E, Mirra SS, Nelson PT, Schneider JA, Thal DR, Trojanowski JQ, Vinters HV, Hyman BT. National Institute on A, Alzheimer's A. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol*. 2012b; 123(1):1–11. [PubMed: 22101365]
- Murray ME, Cannon A, Graff-Radford NR, Liesinger AM, Rutherford NJ, Ross OA, Duara R, Carrasquillo MM, Rademakers R, Dickson DW. Differential clinicopathologic and genetic features of late-onset amnesic dementias. *Acta Neuropathol*. 2014
- Nag S, Yu L, Capuano AW, Wilson RS, Leurgans SE, Bennett DA, Schneider JA. Hippocampal sclerosis and TDP-43 pathology in aging and Alzheimer's Disease. *Ann Neurol*. 2015
- Nelson PT, Abner EL, Scheff SW, Schmitt FA, Kryscio RJ, Jicha GA, Smith CD, Patel E, Markesbery WR. Alzheimer's-type neuropathology in the precuneus is not increased relative to other areas of neocortex across a range of cognitive impairment. *Neuroscience letters*. 2009a; 450(3):336–9. [PubMed: 19010392]
- Nelson PT, Abner EL, Schmitt FA, Kryscio RJ, Jicha GA, Santacruz K, Smith CD, Patel E, Markesbery WR. Brains with medial temporal lobe neurofibrillary tangles but no neuritic amyloid plaques are a diagnostic dilemma but may have pathogenetic aspects distinct from Alzheimer disease. *Journal of neuropathology and experimental neurology*. 2009b; 68(7):774–84. [PubMed: 19535994]
- Nelson PT, Abner EL, Schmitt FA, Kryscio RJ, Jicha GA, Smith CD, Davis DG, Poduska JW, Patel E, Mendiondo MS, Markesbery WR. Modeling the association between 43 different clinical and pathological variables and the severity of cognitive impairment in a large autopsy cohort of elderly persons. *Brain Pathol*. 2010a; 20(1):66–79. [PubMed: 19021630]
- Nelson PT, Alafuzoff I, Bigio EH, Bouras C, Braak H, Cairns NJ, Castellani RJ, Crain BJ, Davies P, Del Tredici K, Duyckaerts C, Frosch MP, Haroutunian V, Hof PR, Hulette CM, Hyman BT, Iwatsubo T, Jellinger KA, Jicha GA, Kovari E, Kukull WA, Leverenz JB, Love S, Mackenzie IR, Mann DM, Masliah E, McKee AC, Montine TJ, Morris JC, Schneider JA, Sonnen JA, Thal DR, Trojanowski JQ, Troncoso JC, Wisniewski T, Woltjer RL, Beach TG. Correlation of Alzheimer

disease neuropathologic changes with cognitive status: a review of the literature. *Journal of neuropathology and experimental neurology*. 2012; 71(5):362–81. [PubMed: 22487856]

- Nelson PT, Estus S, Abner EL, Parikh I, Malik M, Neltner JH, Ighodaro E, Wang WX, Wilfred BR, Wang LS, Kukull WA, Nandakumar K, Farman ML, Poon WW, Corrada MM, Kawas CH, Cribbs DH, Bennett DA, Schneider JA, Larson EB, Crane PK, Valladares O, Schmitt FA, Kryscio RJ, Jicha GA, Smith CD, Scheff SW, Sonnen JA, Haines JL, Pericak-Vance MA, Mayeux R, Farrer LA, Van Eldik LJ, Horbinski C, Green RC, Gearing M, Poon LW, Kramer PL, Woltjer RL, Montine TJ, Partch AB, Rajic AJ, Richmire K, Monsell SE, Schellenberg GD, Fardo DW. Alzheimer' Disease Genetic C. ABCC9 gene polymorphism is associated with hippocampal sclerosis of aging pathology. *Acta Neuropathol*. 2014; 127(6):825–43. [PubMed: 24770881]
- Nelson PT, Head E, Schmitt FA, Davis PR, Neltner JH, Jicha GA, Abner EL, Smith CD, Van Eldik LJ, Kryscio RJ, Scheff SW. Alzheimer's disease is not "brain aging": neuropathological, genetic, and epidemiological human studies. *Acta Neuropathol*. 2011a; 121(5):571–87. [PubMed: 21516511]
- Nelson PT, Jicha GA, Schmitt FA, Liu H, Davis DG, Mendiondo MS, Abner EL, Markesbery WR. Clinicopathologic correlations in a large Alzheimer disease center autopsy cohort: neuritic plaques and neurofibrillary tangles "do count" when staging disease severity. *Journal of neuropathology and experimental neurology*. 2007; 66(12):1136–46. [PubMed: 18090922]
- Nelson PT, Schmitt FA, Jicha GA, Kryscio RJ, Abner EL, Smith CD, Van Eldik LJ, Markesbery WR. Association between male gender and cortical Lewy body pathology in large autopsy series. *J Neurol*. 2010b
- Nelson PT, Schmitt FA, Lin Y, Abner EL, Jicha GA, Patel E, Thomason PC, Neltner JH, Smith CD, Santacruz KS, Sonnen JA, Poon LW, Gearing M, Green RC, Woodard JL, Van Eldik LJ, Kryscio RJ. Hippocampal sclerosis in advanced age: clinical and pathological features. *Brain*. 2011b; 134(Pt 5):1506–18. [PubMed: 21596774]
- Nelson PT, Smith CD, Abner EL, Wilfred BJ, Wang WX, Neltner JH, Baker M, Fardo DW, Kryscio RJ, Scheff SW, Jicha GA, Jellinger KA, Van Eldik LJ, Schmitt FA. Hippocampal sclerosis of aging, a prevalent and high-morbidity brain disease. *Acta Neuropathol*. 2013; 126(2):161–77. [PubMed: 23864344]
- Nelson PT, Wang WX, Partch AB, Monsell SE, Valladares O, Ellingson SR, Wilfred BR, Naj AC, Wang LS, Kukull WA, Fardo DW. Reassessment of risk genotypes (GRN, TMEM106B, and ABCC9 variants) associated with hippocampal sclerosis of aging pathology. *Journal of neuropathology and experimental neurology*. 2015; 74(1):75–84. [PubMed: 25470345]
- Neltner JH, Abner EL, Baker S, Schmitt FA, Kryscio RJ, Jicha GA, Smith CD, Hammack E, Kukull WA, Brenowitz WD, Van Eldik LJ, Nelson PT. Arteriolosclerosis that affects multiple brain regions is linked to hippocampal sclerosis of ageing. *Brain*. 2014; 137(Pt 1):255–67. [PubMed: 24271328]
- Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McCluskey LF, Miller BL, Masliah E, Mackenzie IR, Feldman H, Feiden W, Kretschmar HA, Trojanowski JQ, Lee VM. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science (New York, NY)*. 2006; 314(5796):130–3.
- Pantoni L, Garcia JH, Brown GG. Vascular pathology in three cases of progressive cognitive deterioration. *J Neurol Sci*. 1996; 135(2):131–9. [PubMed: 8867069]
- Poon LW, Clayton GM, Martin P, Johnson MA, Courtenay BC, Sweaney AL, Merriam SB, Pless BS, Thielman SB. The Georgia Centenarian Study. *Int J Aging Hum Dev*. 1992; 34(1):1–17. [PubMed: 1737657]
- Poon, LW.; Jazwinski, SM.; Green, RC.; Woodard, JL.; Martin, P.; Rodgers, WL.; Johnson, MA.; Hausman, DB.; Arnold, J.; Davey, A.; Batzer, MA.; Markesbery, W.; Gearing, M.; Siegler, IC.; Reynolds, S.; Dai, J. Methodological considerations in studying centenarians: Lessons learned from the Georgia Centenarian Studies. In: Poon, LW.; Perls, TT., editors. *Annual Review of Gerontology and Geriatrics: Vol 27 Biopsychosocial approaches to longevity*. Springer; New York: 2007. p. 231–64.
- Rademakers R, Eriksen JL, Baker M, Robinson T, Ahmed Z, Lincoln SJ, Finch N, Rutherford NJ, Crook RJ, Josephs KA, Boeve BF, Knopman DS, Petersen RC, Parisi JE, Caselli RJ, Wszolek ZK,

- Uitti RJ, Feldman H, Hutton ML, Mackenzie IR, Graff-Radford NR, Dickson DW. Common variation in the miR-659 binding-site of GRN is a major risk factor for TDP43-positive frontotemporal dementia. *Hum Mol Genet.* 2008; 17(23):3631–42. [PubMed: 18723524]
- Richmond RL, Law J, KayLambkin F. Morbidity profiles and lifetime health of Australian centenarians. *Australasian journal on ageing.* 2012; 31(4):227–32. [PubMed: 23252980]
- Riley KP, Snowden DA, Markesbery WR. Alzheimer's neurofibrillary pathology and the spectrum of cognitive function: findings from the Nun Study. *Annals of neurology.* 2002; 51(5):567–77. [PubMed: 12112102]
- Robert L, Fulop T. Longevity and its regulation: centenarians and beyond. *Interdisciplinary topics in gerontology.* 2014; 39:198–211. [PubMed: 24862022]
- Rutherford NJ, Carrasquillo MM, Li M, Bisceglia G, Menke J, Josephs KA, Parisi JE, Petersen RC, Graff-Radford NR, Younkin SG, Dickson DW, Rademakers R. TMEM106B risk variant is implicated in the pathologic presentation of Alzheimer disease. *Neurology.* 2012; 79(7):717–8. [PubMed: 22855871]
- Saing T, Dick MC, Nelson PT, Kim RC, Cribbs DH, Head E. Frontal Cortex Neuropathology in Dementia Pugilistica. *J Neurotrauma.* 2011
- Savva GM, Wharton SB, Ince PG, Forster G, Matthews FE, Brayne C. Age, neuropathology, and dementia. *N Engl J Med.* 2009; 360(22):2302–9. [PubMed: 19474427]
- Scheff SW, Neltner JH, Nelson PT. Is synaptic loss a unique hallmark of Alzheimer's disease? *Biochemical pharmacology.* 2014
- Schmitt FA, Davis DG, Wekstein DR, Smith CD, Ashford JW, Markesbery WR. "Preclinical" AD revisited: neuropathology of cognitively normal older adults. *Neurology.* 2000; 55(3):370–6. [PubMed: 10932270]
- Schmitt FA, Nelson PT, Abner E, Scheff S, Jicha GA, Smith C, Cooper G, Mendiondo M, Danner DD, Van Eldik LJ, Caban-Holt A, Lovell MA, Kryscio RJ. University of Kentucky Sanders-Brown healthy brain aging volunteers: donor characteristics, procedures and neuropathology. *Curr Alzheimer Res.* 2012; 9(6):724–33. [PubMed: 22471862]
- Schmitt FA, Wetherby MM, Wekstein DR, Dearth CM, Markesbery WR. Brain donation in normal aging: procedures, motivations, and donor characteristics from the Biologically Resilient Adults in Neurological Studies (BRAiNS) Project. *Gerontologist.* 2001; 41(6):716–22. [PubMed: 11723339]
- Serrano-Pozo A, Qian J, Monsell SE, Frosch MP, Betensky RA, Hyman BT. Examination of the clinicopathologic continuum of Alzheimer disease in the autopsy cohort of the National Alzheimer Coordinating Center. *Journal of neuropathology and experimental neurology.* 2013; 72(12):1182–92. [PubMed: 24226270]
- Shaw K, Gearing M, Davey A, Burgess M, Poon LW, Martin P, Green RC. Successful Recruitment of Centenarians for Post-Mortem Brain Donation: Results from the Georgia Centenarian Study. *The journal of bioscience and medicine.* 2012; 2(1)
- Silver MH, Newell K, Brady C, Hedley-White ET, Perls TT. Distinguishing between neurodegenerative disease and disease-free aging: correlating neuropsychological evaluations and neuropathological studies in centenarians. *Psychosom Med.* 2002; 64(3):493–501. [PubMed: 12021423]
- Singh PP, Singh M, Mastana SS. APOE distribution in world populations with new data from India and the UK. *Annals of human biology.* 2006; 33(3):279–308. [PubMed: 17092867]
- Smith DH, Johnson VE, Stewart W. Chronic neuropathologies of single and repetitive TBI: substrates of dementia? *Nature reviews Neurology.* 2013; 9(4):211–21. [PubMed: 23458973]
- Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR. Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. *Jama.* 1997; 277(10):813–7. [PubMed: 9052711]
- Sonnen JA, Santa Cruz K, Hemmy LS, Woltjer R, Leverenz JB, Montine KS, Jack CR, Kaye J, Lim K, Larson EB, White L, Montine TJ. Ecology of the aging human brain. *Archives of neurology.* 2011; 68(8):1049–56. [PubMed: 21825242]
- Thal DR, Capetillo-Zarate E, Del Tredici K, Braak H. The development of amyloid beta protein deposits in the aged brain. *Sci Aging Knowledge Environ.* 2006; 2006(6):re1. [PubMed: 16525193]

- Toledo JB, Arnold SE, Raible K, Brettschneider J, Xie SX, Grossman M, Monsell SE, Kukull WA, Trojanowski JQ. Contribution of cerebrovascular disease in autopsy confirmed neurodegenerative disease cases in the National Alzheimer's Coordinating Centre. *Brain*. 2013; 136(Pt 9):2697–706. [PubMed: 23842566]
- Tyas SL, Snowdon DA, Desrosiers MF, Riley KP, Markesbery WR. Healthy ageing in the Nun Study: definition and neuropathologic correlates. *Age Ageing*. 2007; 36(6):650–5. [PubMed: 17906306]
- von Gunten A, Ebbing K, Imhof A, Giannakopoulos P, Kovari E. Brain aging in the oldest-old. *Curr Gerontol Geriatr Res*. 2010
- Wang Y, Hashizume Y, Yoshida M, Inagaki T, Kameyama T. Pathological changes of the spinal cord in centenarians. *Pathology international*. 1999; 49(2):118–24. [PubMed: 10355964]
- White L. Brain lesions at autopsy in older Japanese-American men as related to cognitive impairment and dementia in the final years of life: a summary report from the Honolulu-Asia aging study. *J Alzheimers Dis*. 2009; 18(3):713–25. [PubMed: 19661625]
- Wolf DS, Gearing M, Snowdon DA, Mori H, Markesbery WR, Mirra SS. Progression of regional neuropathology in Alzheimer disease and normal elderly: findings from the Nun study. *Alzheimer disease and associated disorders*. 1999; 13(4):226–31. [PubMed: 10609672]
- Zarow C, Weiner MW, Ellis WG, Chui HC. Prevalence, laterality, and comorbidity of hippocampal sclerosis in an autopsy sample. *Brain and behavior*. 2012; 2(4):435–42. [PubMed: 22950047]

Highlights

- Alzheimer's disease pathology was not universal in this sample of extremely old individuals
- Neurofibrillary tangles (e.g., primary age-related tauopathy, PART) was universal
- Lewy body pathology was seen in 16.9% of cases
- Hippocampal sclerosis of aging (HS-Aging) was seen in 20.8% of cases but no frontotemporal lobar degeneration pathology was observed
- *ABCC9* genotype is associated with altered risk for arteriolosclerosis and HS-Aging pathologies

Figure 1. Actuarial tables from the United States Social Security Administration to depict the expected number of persons alive per 100,000

The four curves represent data from 1950 (male blue, female gray), and predicted results for the year 2020 (male orange, female yellow) in the United States. Note that although females live longer than males, the effect of the birth cohort is even stronger than gender. There is a dramatic increase in survival between the years 1950 and 2020. However, those reaching the age of 102 years in both groups – whether male or female – comprise under 1% of individuals. These data indicate that social and medical changes are increasing survival for many, but centenarians will constitute the outer limits of human lifespan for the near future. The website to describe methodology is: http://www.socialsecurity.gov/OACT/NOTES/as120/LifeTables_Body.html

Figure 2. Distribution of density of neuritic amyloid plaques, a defining pathologic hallmark of Alzheimer's disease (AD), in the three cohorts

Neuritic plaques are graded according to the CERAD system (Mirra, 1997). Note that in all three cohorts – Georgia Centenarian Study (Poon, et al., 1992), The Nun Study (Snowdon, et al., 1997, Tyas, et al., 2007), and the University of Kentucky Alzheimer's Disease center (Schmitt, et al., 2012) autopsy cohorts – there is a sizeable minority of individuals that have "Low" or "None" neuritic amyloid plaques. These results seem to indicate that AD pathology, as operationalized by the presence of neuritic amyloid plaques, is not inevitable with brain aging.

Figure 3. The prevalence of three different pathologic combinations in the brains with both Braak staging and CERAD information

Note that some degree of AD-type pathology (NFT+/NP+) is quite prevalent (79%) of cases, followed by "NFT+/NP-", or primary age-related tauopathy (PART) cases (21%). By contrast, there were no "plaque-only" cases observed.

Cases grouped by
number of comorbid
brain pathologies
(# cases in each group,
total n=77)

- 0 (n=3)
- 1 (n=23)
- 2 (n=30)
- 3 (n=13)
- 4 (n=5)
- ≥ 5 (n=3)

Figure 4. The majority of individuals had more than one subtype of comorbid brain pathologies
For the overall study cohort (n=77), the individuals were binned according to the number of different subtypes of pathology in the brain -- Braak NFT stage>II, Cerebral amyloid angiopathy, arteriolosclerosis, HS-Aging or TDP-43 pathology, Lewy body disease, or large infarcts. For example, there were 30 cases with two different comorbid pathologies noted, and 23 cases with only one noted, etc. Note that more than half of the included subjects' brains harbored more than one of these pathologic features.

Figure 5. Cerebrovascular disease pathologies as scored using semi-quantitative measurements
Photomicrographs depicting frontal cortex (Brodmann Area 9) white matter are shown (A–H). Normal white matter vessel (A), compared to mild (B), moderate (C), and severe arteriolosclerosis (D). Virchow Robin space spectrum ranges from 1+ (E), 2+ (F), to 3+ (G). Pigment laden macrophages (H) are also present in the perivascular spaces. Panels A through G at 10x magnification (bar 100 μm); Panel H at 20x magnification (bar 50 μm)

Figure 6. Cerebrovascular disease pathologies in 22 brain areas
A convenience sample of 49 cases of the Georgia Centenarian Study were scored using semi-quantitative measurements. The schematic shows (from top to bottom of the figure) the ventral surface, medial aspect, and lateral brain convexity to help visualize the neuroanatomical locations. Separate data are shown for arteriolosclerosis (blue), extravascular pigmented macrophages (red), and expanded Virchow-Robin spaces (green). Each was scored on a four-tier (0–3) scale, from low to high severity, in each case.

Table 1

Cohorts with clinical and apolipoprotein E (*APOE*) information

Cohort	N, overall	Centenarians included, N	Average age death, overall	Age of death Range	M/F*	Avg Final MMSE*, +/- stdev	<i>APOE</i> ε4* allele (/ genotyped)
Georgia Centenarian Study	49	41	102.0	98–107	6/43	13.7 +/- 9.3	8/48
The Nun Study	17	17	102.2	100–107	0/17	16.2 +/- 8.9	1/17
U. Kentucky ADC	11	11	101.3	100–105	2/9	22.1 +/- 6.5	2/11
Total/(Overall)	77	69	102.0	98–107	8/69	15.4 +/- 9.2	11/76

* M/F: Male/Female; MMSE: Folstein Mini-Mental State Examination (Folstein, et al., 1975); *APOE* ε4: refers to the proportion of genotyped cases that had one Apolipoprotein E ε4 allele (no subject had two ε4 alleles).

Alzheimer’s disease-type pathological changes, showing Braak NFT stages and CERAD neuritic amyloid plaque grading, for the combined study cohort

Table 2

		Braak Neurofibrillary Tangle Stages							
		0	I	II	III	IV	V	VI	ND*
CERAD Neuritic amyloid plaque grading	None	0	3	7	5	0	0	0	1
	Sparse	0	0	4	1	2	3	2	1
	Moderate/Severe	0	2	10	8	8	12	5	3
			5	21	14	10	15	7	5
									77

* ND indicates that Braak staging was not performed, usually because of the presence of severe hippocampal sclerosis precluding reliable Braak staging

Table 3

Lewy body pathological changes in the cohorts, by Lewy body disease subtype (McKeith, et al., 2004).

Cohort	N, overall	Lewy Body disease		
		Brainstem predominant type	Limbic type	Neocortical/diffuse type
Georgia Centenarian Study	49	0	7	3
The Nun Study	17	0	1	0
U. Kentucky ADC	11	0	1	1
Total/(Overall)	77	0	9	4

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Hippocampal sclerosis of aging (HS-Aging, diagnosed on hematoxylin and eosin stains), and TDP-43 immunoreactive pathology, by cohort to show combinations of pathology

Table 4

Cohort	N, overall	N, HS-Aging	N, TDP-43 assessed	N, HS+/TDP+	N, HS+/TDP-	N, HS-/TDP+
Georgia Centenarian Study	49	6	43	6	0	8
The Nun Study	17	6	17	4	2	0
U. Kentucky ADC	11	4	8	2	1	1
Total	77	16	68	12	3	9

Table 5

Average arteriolosclerosis scores from all 22 brain regions analyzed from the Georgia Centenarians, stratifying separately by HS-Aging (HS+/-), TDP-43 (TDP+/-), and Alzheimer's disease (AD+/-) pathologies

	HS+	HS-	TDP+	TDP-	AD+	AD-
	n=6	n=43	n=14	n=35	n=14	n=35
Counted neocortical NFTs*, Avg	24.4	8.1	16.2	7.6	29.0	2.5
Average Arteriolosclerosis Score						
Lt frontal pole	1.40	0.93	1.09	0.96	1.00	1.00
Lt frontal cortex (BA 9)	1.33	1.16	1.43	1.09	1.29	0.93
Lt sup/mid temporal cortex (BA 21/22)	1.50	1.00	1.43	0.91	1.03	1.15
Lt hippocampus	0.83	0.52	0.57	0.56	0.56	0.57
Rt hippocampus	1.33	0.49	0.71	0.55	0.55	0.71
Lt entorhinal cortex	1.00	0.43	0.71	0.41	0.41	0.71
Rt entorhinal cortex	0.67	0.53	0.43	0.60	0.54	0.57
Lt amygdala	1.50	1.02	1.07	1.09	1.11	1.00
Rt amygdala	2.00	1.12	1.33	1.18	1.22	1.20
Lt parietal cortex (BA 39/40)	1.33	1.26	1.50	1.17	1.14	1.57
Lt occipital cortex (BA 17/18)	1.17	1.07	1.21	1.03	1.09	1.07
Lt basal ganglia	1.67	1.49	1.57	1.49	1.49	1.57
Midbrain	0.67	0.51	0.50	0.54	0.54	0.50
Pons	1.00	0.42	0.79	0.37	0.46	0.57
Medulla oblongata	1.00	0.53	0.62	0.57	0.71	0.29
Dentate (deep cerebellar) nucleus	1.33	1.21	1.29	1.20	1.23	1.21
Cerebellar Vermis	0.33	0.07	0.14	0.09	0.14	0.00
Lt temporal pole	1.17	1.38	1.69	1.23	1.20	1.77
Lt thalamus	1.17	0.98	0.93	1.03	1.00	1.00
Lt anterior cingulate	2.00	1.31	1.86	1.21	1.29	1.64
Lt posterior cingulate	1.50	0.88	1.07	0.91	0.91	1.07
Lt insula	1.60	1.46	1.69	1.39	1.37	1.71

	HS+	HS-	TDP+	TDP-	AD+	AD-
	n=6	n=43	n=14	n=35	n=14	n=35
Overall Arteriolosclerosis avg	1.25	0.90	1.07	0.89	0.92	0.99
p value **	0.0011		0.014		0.52	

* Neocortical NFT counts refer to summed NFTs counted from frontal, temporal, parietal, and occipital cortices in each case, as previously described (Nelson, et al., 2007).

** Statistics refer to comparisons between pathology groups for mean number of brain regions with presence of at least moderate arteriolosclerosis versus absent or mild severity; see Methods.

Table 6

Average arteriolosclerosis scores from all 22 brain regions analyzed from the Georgia Centenarians (n=45 with genetic information available), stratifying by *ABCC9* rs704178 genotype

	<i>ABCC9</i> rs704178 genotype		
	C_C (n=9)	G_C (n=26)	G_G (n=10)
# Cases with HS-Aging pathology	0	3	2
	Average Arteriolosclerosis Score		
Lt frontal pole	0.80	1.05	0.86
Lt frontal cortex (BA 9)	1.00	1.27	1.00
Lt sup/mid temporal cortex (BA 21/22)	0.78	1.16	1.00
Lt hippocampus	0.50	0.54	0.50
Rt hippocampus	0.38	0.58	0.78
Lt entorhinal cortex	0.44	0.48	0.50
Rt entorhinal cortex	0.11	0.65	0.60
Lt amygdala	0.67	1.23	1.10
Rt amygdala	0.80	1.20	1.50
Lt parietal cortex (BA 39/40)	1.00	1.35	1.40
Lt occipital cortex (BA 17/18)	0.67	1.38	0.80
Lt basal ganglia	1.56	1.38	1.90
Midbrain	0.56	0.42	0.60
Pons	0.33	0.42	0.60
Medulla oblongata	0.67	0.44	0.80
Dentate (deep cerebellar) nucleus	1.00	1.23	1.50
Cerebellar Vermis	0.22	0.00	0.10
Lt temporal pole	1.13	1.35	1.50
Lt thalamus	1.00	1.46	1.67
Lt anterior cingulate	0.56	1.04	1.10
Lt posterior cingulate	0.89	0.92	1.20
Lt insula	1.44	1.36	1.88
Overall Arteriosclerosis avg	0.75	0.95	1.04
p value *, GG vs (GC+CC)	0.026		
p value *, GG vs (GC+CC) without HS-Aging cases	0.011		
p value *, GG vs CC	0.007		

* Statistics refer to comparisons between genotype groups for mean number of brain regions with presence of at least moderate arteriolosclerosis versus absent or mild severity; see Methods.