doi:10.1093/brain/awt318 Brain 2014: 137; 255–267 | 255

BRAIN
A JOURNAL OF NEUROLOGY

Arteriolosclerosis that affects multiple brain regions is linked to hippocampal sclerosis of ageing

Janna H. Neltner,[1] Erin L. Abner,[2] Steven Baker,[2] Frederick A. Schmitt,[2,3] Richard J. Kryscio,[2,4] Gregory A. Jicha,[2,3] Charles D. Smith,[2,3] Eleanor Hammack, Walter A. Kukull,[5,6] Willa D. Brenowitz,[5,6] Linda J. Van Eldik[2,7] and Peter T. Nelson[1,2]

1 Department of Pathology, Division of Neuropathology, University of Kentucky, Lexington, KY 40536, USA
2 Sanders-Brown Centre on Ageing, University of Kentucky, Lexington, KY 40536, USA
3 Department of Neurology, University of Kentucky, Lexington, KY 40536, USA
4 Department of Statistics, University of Kentucky, Lexington, KY 40536, USA
5 National Alzheimer's Coordinating Centre, University of Washington, Seattle, WA 98195, USA
6 Department of Epidemiology, University of Washington, Seattle, WA. 98195, USA
7 Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536, USA

Correspondence to: Peter T. Nelson, MD, PhD,
Department of Pathology
Division of Neuropathology and the Sanders-Brown Centre on Ageing, Rm 311, Sanders-Brown Building, 800 S. Limestone, University of Kentucky, Lexington, KY 40536-0230, USA
E-mail: pnels2@email.uky.edu

Hippocampal sclerosis of ageing is a prevalent brain disease that afflicts older persons and has been linked with cerebrovascular pathology. Arteriolosclerosis is a subtype of cerebrovascular pathology characterized by concentrically thickened arterioles. Here we report data from multiple large autopsy series (University of Kentucky Alzheimer's Disease Centre, Nun Study, and National Alzheimer's Coordinating Centre) showing a specific association between hippocampal sclerosis of ageing pathology and arteriolosclerosis. The present analyses incorporate 226 cases of autopsy-proven hippocampal sclerosis of ageing and 1792 controls. Case–control comparisons were performed including digital pathological assessments for detailed analyses of blood vessel morphology. We found no evidence of associations between hippocampal sclerosis of ageing pathology and lacunar infarcts, large infarcts, Circle of Willis atherosclerosis, or cerebral amyloid angiopathy. Individuals with hippocampal sclerosis of ageing pathology did not show increased rates of clinically documented hypertension, diabetes, or other cardiac risk factors. The correlation between arteriolosclerosis and hippocampal sclerosis of ageing pathology was strong in multiple brain regions outside of the hippocampus. For example, the presence of arteriolosclerosis in the frontal cortex (Brodmann area 9) was strongly associated with hippocampal sclerosis of ageing pathology ($P < 0.001$). This enables informative evaluation of anatomical regions outside of the hippocampus. To assess the morphology of brain microvasculature far more rigorously than what is possible using semi-quantitative pathological scoring, we applied digital pathological (Aperio ScanScope) methods on a subsample of frontal cortex sections from hippocampal sclerosis of ageing ($n = 15$) and control ($n = 42$) cases. Following technical studies to optimize immunostaining methods for small blood vessel visualization, our analyses focused on sections immunostained for smooth muscle actin (a marker of arterioles) and CD34 (an endothelial marker), with separate analyses on grey and white matter. A total of 43 834 smooth muscle actin-positive vascular profiles and 603 798 CD34-positive vascular profiles were evaluated. In frontal cortex of cases with hippocampal sclerosis of ageing, smooth muscle actin-immunoreactive arterioles had thicker walls ($P < 0.05$), larger perimeters ($P < 0.03$), and larger vessel areas ($P < 0.03$) than controls. Unlike the arterioles,

Received June 11, 2013. Revised September 13, 2013. Accepted September 23, 2013. Advance Access publication November 21, 2013

CD34-immunoreactive capillaries had dimensions that were unchanged in cases with hippocampal sclerosis of ageing versus controls. Arteriolosclerosis appears specific to hippocampal sclerosis of ageing brains, because brains with Alzheimer's disease pathology did not show the same morphological alterations. We conclude that there may be a pathogenetic change in aged human brain arterioles that impacts multiple brain areas and contributes to hippocampal sclerosis of ageing.

Keywords: TDP-43; NACC; FTLD; SMA; HS-Ageing

Abbreviations: FTLD = frontotemporal lobar degeneration; HS-Ageing = hippocampal sclerosis of ageing; NACC = National Alzheimer's Disease Coordinating Centre; SMA = smooth muscle actin; TDP-43 = TAR DNA binding protein 43; UK-ADC = University of Kentucky Alzheimer's Disease Centre

Introduction

Hippocampal sclerosis of ageing (HS-Ageing) is a poorly-understood brain disease with an enormous impact on public health. Currently the disease is defined by pathology: HS-Ageing is characterized by cell loss, gliosis and atrophy in the hippocampal formation that is out of proportion with Alzheimer's disease pathology in the same structures (Jellinger, 1994; Amador-Ortiz and Dickson, 2008; Nelson *et al.*, 2011; Montine *et al.*, 2012; Zarow *et al.*, 2012). Here we apply the definition of 'hippocampal formation' to include the subiculum (Amaral and Lavenex, 2007), which is often severely affected in HS-Ageing. Hippocampal sclerosis pathology can be associated with different underlying causes, and the term 'HS-Ageing' refers to a specific subset with advanced age (Nelson *et al*., 2011, 2013). Even when co-morbid brain pathologies are taken into account, the presence of HS-Ageing pathology at autopsy correlates independently with substantial cognitive impairment (Kuslansky *et al.*, 2004; Nelson *et al.*, 2010, 2011). HS-Ageing pathology is especially prevalent in brains of the 'oldest old', affecting ~10–30% of individuals over the age of 85 years (Leverenz and Lipton, 2008; Nelson *et al*., 2011, 2013; Corrada *et al.*, 2012).

The molecular pathogenesis of HS-Ageing is not well understood (Nelson *et al*., 2013). One aetiological mechanism that may cause or worsen HS-Ageing pathology is cerebrovascular disease (Dickson *et al.*, 1994; Zarow *et al.*, 2008). Impairment of blood supply to the brain with reperfusion can cause death of hippocampal neurons, although this pathology is not immunopositive for aberrant TAR-DNA binding protein 43 (TDP-43) pathology (Neumann *et al.*, 2006), as cases with HS-Ageing tend to be (Amador-Ortiz *et al.*, 2007; Lee *et al.*, 2008; Nelson *et al*., 2011). Further, some degree of cerebrovascular pathology is near-universal in individuals >90 years of age (Garde *et al.*, 2000; Polvikoski *et al.*, 2010; Nelson *et al*., 2011). However, we previously found no strong and specific correlation between large-vessel cerebrovascular pathology and HS-Ageing pathology after age adjustment in the largest HS-Ageing autopsy series to date (Nelson *et al*., 2011). A more complete assessment of cerebrovascular pathology would involve additional data sets along with a rigorous assessment of small vessel pathologies.

Here we analysed data drawn from multiple autopsy series to test rigorously the association between cerebrovascular pathology and HS-Ageing. We report a strong tendency for pathologically confirmed arteriolosclerosis to be associated with HS-Ageing pathology in the University of Kentucky Alzheimer's Disease Centre (UK-ADC) autopsy series, and this finding was confirmed in two additional autopsy series drawn from the National Alzheimer's Coordinating Centre (NACC) and the Nun Study. Arteriolosclerosis is a subtype of cerebrovascular pathology characterized by concentrically thickened and/or dysmorphic small blood vessels, particularly arterioles. Because the operational definition of arteriolosclerosis is vague, we applied digital pathological methods for rigorous assessment of small vessel histomorphology in a subsample of UK-ADC cases. We found that neither non-arteriolosclerosis cerebrovascular pathology subtypes nor cardiovascular risk factors appear to be associated with HS-Ageing pathology (as arteriolosclerosis is), which may indicate a specific role of arteriolosclerosis in HS-Ageing.

Materials and methods

Research volunteers who had come to autopsy from UK-ADC ($n = 327$) (Nelson *et al.*, 2007; Schmitt *et al.*, 2012), NACC neuropathology data set excluding UK-ADC cases ($n = 1444$) (Beekly *et al.*, 2004, 2007), and Nun Study ($n = 247$) (Snowdon *et al.*, 1996; Wolf *et al.*, 1999; Riley *et al.*, 2002; Mortimer, 2012) cohorts were the basis for the study. All assessments were performed with institutional review board approval from their respective institutions. Clinicians and study staff lacked knowledge about the character or severity of pathology in the regions studied, minimizing bias in terms of case selection that would have affected our main outcome measures. All included subjects were at least 80 years old at death and were free from Parkinson's disease, autopsy-determined prion disease, triplet repeat diseases, brain cancer, frontotemporal lobar degeneration (FTLD), genetic or chromosomal abnormalities, or another neurological disease that might explain a dementia syndrome.

Among UK-ADC cases, all autopsied subjects with detailed quantitative neuropathological data were initially considered for inclusion ($n = 660$). Cases younger than 80 years at the time of death ($n = 208$), missing global ratings of arteriolosclerosis ($n = 100$) or over 80 years at death with any of the pathologies indicated above ($n = 25$) were excluded. Global ratings of arteriolosclerosis were missing for a portion of the sample: 9/48 (18.8%) cases with HS-Ageing and 91/379 (24.0%) cases without HS-Ageing pathology. These cases were excluded leaving 39 cases with HS-Ageing and 288 cases without HS-Ageing. For more information about cases missing arteriolosclerosis ratings both with and without HS-Ageing pathology, see Supplementary Table 1.

Neuropathological evaluations were as described previously (Nelson *et al.*, 2009). For both the UK-ADC and NACC cohorts,

semi-quantitative global neuropathological assessments of arteriolosclerosis were performed on a four-tier categorization system from the NACC Neuropathology Data Element Dictionary (v9.1) addressing the following coded question: 'Is arteriolosclerosis (small parenchymal arteriolar disease) present?', with responses (scored as 0–3) to designate 'none', 'mild', 'moderate' or 'severe', respectively. Additional cerebrovascular pathology measures in the UK-ADC cohort included infarct counts, semi-quantitative ratings of atherosclerosis, cerebral amyloid angiopathy, micro-infarcts, lacunar infarcts, pale infarcts and cortical laminar necrosis as previously described (White *et al.*, 2002; Nelson *et al*., 2007, 2010), as well as region-specific indicators for the presence or absence of arteriolosclerosis.

The Nun Study is a longitudinal study of participants from seven regions in the USA. Cases initially included in the current study ($n = 526$) had available neuropathological data as worked up at the UK-ADC. Exclusion criteria for the Nun Study cases were age <80 years at the time of death ($n = 37$), or any case with missing pathological information or distinct non-Alzheimer's disease dementing syndrome ($n = 31$). Because the Nun Study was used as a validation cohort, also excluded were 211 cases missing detailed data about arteriolosclerosis severity. All exclusions were blind with regard to other aspects of pathological or clinical status (i.e. whether or not HS-Ageing pathology was present). Thus, 247 cases were included in the current study. The Nun Study data were not contributory to the NACC neuropathology data, and the arteriolosclerotic pathology was graded as a dichotomous (as opposed to four-level semi-quantitative) 'global' variable in this data set as to whether it was noted histopathologically in any brain region.

The NACC neuropathology data set contains information on neuropathological features and diagnoses for autopsied individuals who were evaluated at one of 34 different past and present Alzheimer's Disease Centres (ADCs) throughout the USA. This analysis includes subjects 80 years or older at death, who had been autopsied between January 2006 and December 2012. Before 2006, the diagnosis of hippocampal sclerosis pathology in the NACC neuropathology data set was infrequent (data not shown). Subjects were excluded from the NACC data analyses if they were evaluated at the UK-ADC; if they had missing pathological evaluation of neuritic plaque densities or Braak stages for neurofibrillary tangles; and if they had any of the unusual pathologies (e.g. prion disease) indicated above. From this validation cohort of 1631 cases, 187 cases were excluded because of lack of data about arteriolosclerosis pathology. Thus the final NACC cohort included 1444 autopsied cases.

Statistical analysis

Analyses were conducted separately on each cohort. Age at death is a potential confounder because advanced age increases the risk of HS-Ageing pathology and is also associated with higher burden of cerebrovascular pathology (Nelson *et al*., 2011*a*, *b*). Thus, in the UK-ADC cohort analyses, for which the most detailed data were available, three methods were used to control for the effect of age. Generalized linear regression was performed, with age at death used first as a covariate and then split into three categories [80–84 (28%), 85–89 (34%) and $\geqslant$90 (38%)] and used as a factor. Finally, because there was poor overlap in the age distributions at the tails, an age-matched (exact matching on integer ages) nested case–control sample ($n = 151$, 39 cases and 112 controls) with two to three controls per case was selected for further study.

Potential UK-ADC group differences in infarct counts were compared with Poisson regression due to the preponderance of zero counts. Probability of HS-Ageing pathology present given semi-quantitative measures of global atherosclerosis, arteriolosclerosis, and amyloid angiopathy was assessed with logistic regression. Logistic regression was also used to analyse a subset of the sample that had area-specific indications of the presence or absence of arteriolosclerosis.

Medical history data were also available on a subset of the sample, and logistic regression was used to estimate the odds ratio for cases with HS-Ageing versus controls separately for each condition. In the age-matched analyses, conditional regression was used to evaluate the data. Statistical significance for all comparisons was set at $P < 0.05$. The family-wise type I error rate was set at 0.05, and the Bonferroni-Holm method was used to correct for multiple comparisons. Analyses were conducted via SAS 9.3®.

Digital pathology

Because large autopsy cohort data indicated that arteriolosclerosis is associated with HS-Ageing pathology, but the operationalization of arteriolosclerosis is vague (see above), we performed digital pathological assessment of small blood vessels in human brain for more rigorous study of the specific small blood vessel morphological parameters that are associated with HS-Ageing pathology. Technical evaluations were performed using five different potential small blood vessel antibody markers: CD34 (QBEnd 10, Dako), α-SMA (smooth muscle actin; 1A4, Dako), CD31 (JC70A, Dako), factor VIII (polyclonal, Dako), and collagen IV (CIV22, Cell Marque). The initial screen was to assess antibody robustness and appropriateness for banked tissue that had been fixed at various intervals in formalin before paraffin embedding. Adjacent 2-mm thick portions of frontal cortex (Brodmann area 9) were fixed in formalin for six different time intervals (1, 3, 7, 14, 30, and 60 days), before paraffin embedding, sectioning, immunostaining, scanning on the Aperio ScanScope, and image analyses (see below for more details). Results provided support for using CD34 (a marker of capillaries) and α-SMA (a marker of arterioles), as a result of a combination of strong staining intensity over a range of fixation times and excellent immunohistochemical signal to noise ratio (Supplementary Table 2).

For detailed digital image analyses, a convenience sample of cases ($n = 57$) was selected as a representative subsample of the UK-ADC autopsy cohort. End-stage Alzheimer's disease (Braak stage VI) and FTLD cases were excluded because end-stage neurodegenerative pathology would be presumed to induce secondary vascular changes. Cases with neocortical α-synucleinopathy also were excluded. The main criteria for case selection were a sampling of brains with and without HS-Ageing pathology, including distributions of Braak stages (0–5) and arteriolosclerosis severities (0–3) that were similar to the overall cohort (Supplementary Table 3). After the cases were selected, slides were cut from archived paraffin-embedded frontal cortex and stained with CD34 and α-SMA antibodies. Both the CD34 and α-SMA stained slides were then loaded onto the Aperio ScanScope XT and scanned at $\times 40$ magnification to create virtual slides, which were subsequently stored on a dedicated server. All pre-analytical steps were performed blind to pathological diagnosis, and no additional cases were included nor were any cases excluded after the initial case selection.

Image analyses were performed using the Aperio Microvessel Analysis algorithm, which was included with the Image Analysis Toolbox™ software. Modifications were made to the default algorithm parameters to adjust for background staining (Supplementary Table 4). For efficiency, a series of 4 mm^2 regions ('boxes'; Supplementary Fig. 1) were used for quantitation, performed as described previously in detail for other neuropathological quantitation (Neltner *et al.*, 2012).

Briefly, a 4 mm^2 box was placed within the grey matter in the region of highest pathology. Subsequent boxes were scattered through the remaining grey matter at maximum distances from each other to ensure an even distribution throughout the slide (Supplementary Fig. 1). The same protocol was repeated for the white matter. Up to 10 boxes were placed in each cortical region (grey and white matter). The microvessel algorithm was subsequently run on these regions, in both the grey and white matter, for each case. The following parameters were calculated using the modified microvessel algorithm: number of vessels, total analysis area (μm^2), total stain area (μm^2), average stain intensity, microvessel density (vessels/μm^2), as well as mean (with standard deviation) and median vessel areas (μm^2), vessel perimeter (μm), lumen area (μm^2),vascular area (μm^2), and vessel wall thickness (μm). The resulting data were downloaded into spreadsheet format for assessment in Microsoft Excel. For digital pathological analyses, because we were testing the hypothesis that there is a greater burden of arteriolosclerosis in cases with HS-Ageing pathology, we used a one-tailed Student's *t*-test for between-group comparisons.

Results

A central goal of this study was to test whether cerebrovascular changes correlate specifically with HS-Ageing pathology. Previous work (Dickson *et al*., 1994; Jellinger, 1994; Nelson *et al*., 2011) led us to focus on pathological changes in small blood vessels, particularly arteriolosclerosis. Observations using haematoxylin and eosin stains to visualize disease-related pathology and blood vessel changes are shown in Figs 1 and 2. Figure 1 illustrates some of the hippocampal changes seen in HS-Ageing brains versus controls. Figure 2 shows blood vessel profiles at high magnification to illustrate the four-tier scoring system that was used for semi-quantitative assessment of arteriolosclerosis severity in the UK-ADC and NACC autopsy databases; in the Nun Study data, a dichotomous 'global' variable was used to indicate whether appreciable arteriolosclerosis was observed in those brains.

Association between hippocampal sclerosis of ageing pathology and arteriolosclerosis in large autopsy databases

The data from large autopsy series used in the current study represented different cohort types (Table 1) including the UK-ADC (convenience sample; $n = 327$ cases met inclusion criteria), the Nun Study (population-based sample; $n = 247$), and NACC (ADC multicentre sample; $n = 1444$). As shown in Table 2, global arteriolosclerosis (which is a term used to denote a single semi-quantitative parameter of a given brain's arteriolosclerosis, incorporating all areas evaluated) was positively correlated with HS-Ageing pathology in both the Nun Study ($P < 0.04$) and the statistically well-powered NACC autopsy data ($P < 0.0001$). More detailed description of NACC data, stratifying for HS-Ageing and Alzheimer's disease pathology, is presented in Supplementary Table 5. For studies performed at UK-ADC, both sides of the brain were evaluated, but some centres among the NACC cohort evaluated only one side. We note that the age distribution in the Nun Study was right-shifted in the cases with HS-Ageing as

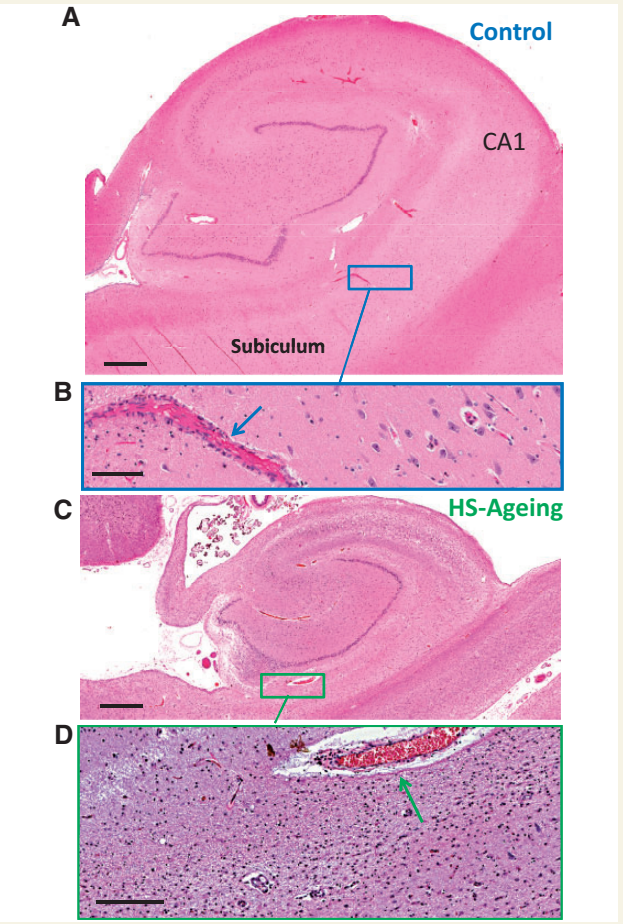

Figure 1 Photomicrographs of haematoxylin and eosin stained human hippocampal sections without (**A** and **B**) and with (**C** and **D**) hippocampal sclerosis of ageing pathology. (**A**) Low-power photomicrograph demonstrates the normal architecture of the hippocampal formation including CA1 and subiculum. (**B**) Inset from **A** shows relatively smooth-walled vessel (blue arrow) and an evenly-stained neuropil with some neurons on the right side of the photomicrograph. (**C**) Low-power photomicrograph depicting HS-Ageing pathology; the hippocampus is shrunken relative to normal (same scale as **A**), with rarefaction of the neuropil and cell loss in CA1 and subiculum. (**C**) Inset from **B** shows higher power photomicrograph with disrupted neuropil lacking the normal cellular contents as shown in **B**. An arteriole is depicted with green arrow. Scale bars: A and C = 1 mm; B and D = 100 mm.

was the case with the UK-ADC cohort, but we performed this analysis initially without age correction other than the inclusion of cases older than 80 years at death, and using a two-tailed *P*-value. When the Nun Study analysis was age-adjusted by logistic regression, then, depending on the specification for age, the one-tailed *P*-value for the correlation between HS-Ageing pathology and arteriolosclerosis is between 0.04 and 0.06 (data not shown). In secondary analyses of NACC data, neither history of hypertension nor diabetes was associated with HS-Ageing pathology (data not shown).

UK-ADC autopsy cohort cases, comprising highly detailed clinical and pathological data (including anatomical region-specific

data), were the main focus in assessing the specificity of the association between HS-Ageing pathology and arteriolosclerosis. Comparison groups were generated related to HS-Ageing pathology and were controlled for age at death through simple covariate adjustment and through age-matching, which makes no assumptions about the relationship (e.g. linear, quadratic) between age at death and neuropathological findings. The age-matched groups, which were used for subsequent analyses in the UK-ADC cohort, are shown in Supplementary Table 6. Both methods used to correct for age yielded similar results. There was no evidence that the distribution of the following cerebrovascular pathology subtypes was unequal between cases and controls: total infarcts, micro-infarcts, pale infarcts, lacunar infarcts, cortical laminar necrosis, circle of Willis atherosclerosis and cerebral amyloid angiopathy (CAA) (Table 3, Supplementary Tables 7 and 8). Of the cerebrovascular pathology subtypes, arteriolosclerosis pathology was more severe in cases with HS-Ageing pathology than controls ($P < 0.03$) although these data would not satisfy the correction for multiple comparisons (Table 3 and Supplementary Tables 7 and 8). Unexpectedly, the rate of haemorrhagic infarction was lower in cases with HS-Ageing pathology in this sample ($P \sim 0.05$), but again this P-value would not survive correction for multiple comparisons; we did not follow up this observation.

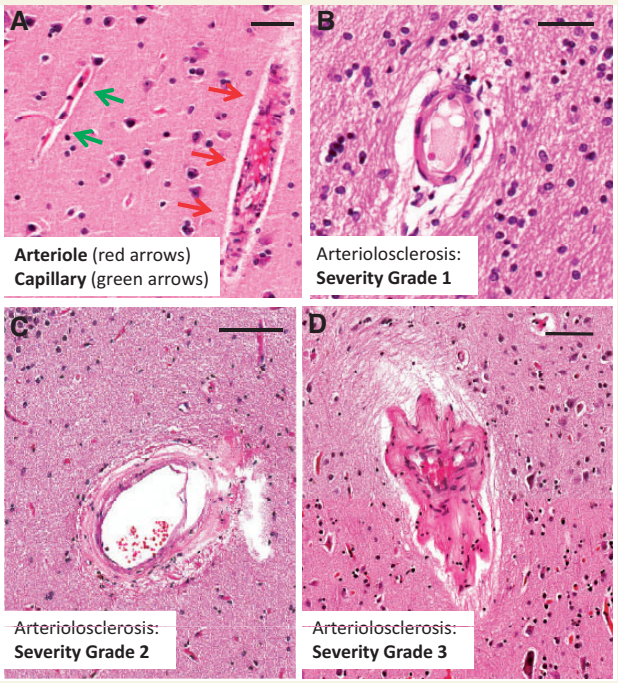

Figure 2 A range of blood vessel changes that can be seen on haematoxylin and eosin stain, along with pathological severity scores for arteriolosclerosis. (**A**) Normal-appearing blood vessels including a capillary profile (green arrows) and what is presumed to be an arteriole (red arrows). (**B**) Grade 1 arteriolosclerosis, with relatively mildly thickened vessel wall and modest apparent disruption of the vessel wall and surrounding neuropil. (**C**) Grade 2 arteriolosclerosis shows more thickening of the arteriolar wall, and in this case there is a tortuous vessel contour, as is frequently seen in cases with widespread arteriolosclerosis. (**D**) Grade 3 arteriolosclerosis is characterized by marked dysmorphic changes that tend to appear to partly occlude the vessel lumen, and can be accompanied by concentric thickening and possible collagenization of the arteriolar wall. Scale bars: **A** and **B** = 50 mm; **C** and **D** = 100 mm.

Arteriolosclerosis has previously been correlated with cardiovascular co-morbidities such as hypertension and diabetes, so we sought to test the associations between known cardiovascular risk factors and HS-Ageing pathology. We assessed the associations between HS-Ageing pathology and the presence/absence of nine different risk factors including hypertension and type II diabetes in both the age-matched and unmatched for age case-control samples (Supplementary Tables 9–11 show similar results after different methods accounting for age). None of these risk factors were independently linked to HS-Ageing pathology in the UK-ADC cohort (95% confidence intervals all included 1.0) although we note limited sample size and potential bias because of missing data (Supplementary Table 1).

In comparing UK-ADC cases with and without HS-Ageing pathology, we asked how anatomically widespread were the differences in arteriolosclerosis pathology. In many different brain regions, including neocortical and subcortical areas, arteriolosclerosis is more severe in cases with HS-Ageing pathology (Table 5). The exact arterial territories are not always possible to obtain when reviewing a microscope slide and thus are not included here. Notably, the hippocampal formation (CA1 and subiculum) was not among the areas where greater arteriolosclerosis was

Table 1 Cohort characteristics

Summary	UK-ADC	Nun Study	NACC
Total cases included, *n*	327	247	1444
With HS-Ageing pathology, *n*	39	30	157
Cohort type	Convenience	Population-based	Multicentre (ADCs)
Age at death (years), mean ± SD	88.0 ± 5.1	90.3 ± 4.8	88.3 ± 5.7
HS-Ageing	90.2 ± 4.6	92.9 ± 5.1	89.1 ± 5.6
No HS-Ageing	87.8 ± 5.1	89.9 ± 4.6	88.2 ± 5.7
% HS-Ageing (no Alzheimer's disease)	4.6	6.5	4.2
% Alzheimer's disease (no HS-Ageing)	45.3	19.8	41.9
% HS-Ageing + Alzheimer's disease	7.3	5.7	6.4

All included participants were at least 80-years-old at the time of death.

Table 2 Arteriolosclerosis versus HS-Ageing in the UK-ADC, Nun Study and NACC cohorts (*P*-value by determined using chi-square test)

	HS-Ageing	No HS-Ageing	*P*
UK-ADC			
Global arteriolosclerosis[a] (*n*, %)	*n* = 39	*n* = 288	
None	3 (7.7)	67 (23.3)	0.018
Mild	22 (56.4)	171 (59.4)	
Moderate	12 (30.8)	44 (15.3)	
Severe	2 (5.1)	6 (2.1)	
Nun Study			
Global arteriolosclerosis[a] (*n*, %)	*n* = 30	*n* = 217	
Absent	20 (66.7)	179 (82.5)	0.04
Present	10 (33.3)	38 (17.5)	
NACC			
Global arteriolosclerosis[a] (*n*, %)	*n* = 157	*n* = 1287	
None	13 (8.3)	274 (21.3)	<0.0001
Mild	69 (43.9)	445 (34.6)	
Moderate	63 (40.1)	387 (30.0)	
Severe	12 (7.6)	181 (14.1)	

[a]Global arteriolosclerosis is a term used to denote a single semi-quantitative parameter of a given brain's arteriolosclerosis, incorporating all areas evaluated.

Table 3 Cerebrovascular pathology stratified by HS-Ageing pathological status in UK-ADC participants (*n* = 327)

	HS-Ageing (*n* = 39)	No HS-Ageing (*n* = 288)	*P*[d]
Infarcts[a]			
Total infarcts	1.79 ± 2.35	1.93 ± 3.08	0.56
Micro-infarcts	0.56 ± 1.05	0.86 ± 1.83	0.14
Pale infarcts	0.62 ± 1.53	0.56 ± 1.48	0.93
Lacunar infarcts	0.46 ± 0.60	0.27 ± 0.71	0.12
Haemorrhagic infarcts	0.13 ± 0.52	0.10 ± 0.39	0.54
Haemorrhages	0.03 ± 0.16	0.13 ± 0.70	0.05
Macro-infarcts	0.33 ± 0.70	0.28 ± 0.71	0.86
Cortical laminar necrosis (*n*, %)[b]			0.99
Present	1 (2.6)	0 (0.0)	
Absent	38 (97.4)	276 (95.8)	
Not assessed	0 (0.0)	12 (4.2)	
Atherosclerosis (Circle of Willis) (*n*, %)[b]			0.77
No visible	0 (0.0)	9 (3.1)	
Scant, patchy	3 (7.7)	50 (17.4)	
All vessels < 50%	9 (23.1)	56 (19.4)	
Any vessel >50%	12 (30.8)	96 (33.3)	
Severe, occlusive	14 (35.9)	73 (25.4)	
Not assessed	1 (2.6)	4 (1.4)	
Amyloid angiopathy (*n*, %)[b]			0.51
None	18 (46.2)	115 (39.9)	
Mild	8 (20.5)	97 (33.7)	
Moderate	10 (25.6)	51 (17.7)	
Severe	3 (7.7)	23 (8.0)	
Not assessed	0 (0.0)	2 (0.7)	
Arteriosclerosis (*n*, %)[b,c]			0.029
None	3 (7.7)	67 (23.3)	
Mild	22 (56.4)	171 (59.4)	
Moderate	12 (30.8)	44 (15.3)	
Severe	2 (5.1)	6 (2.1)	

[a]Results presented are mean ± SD, Poisson regression.
[b]Logistic regression.
[c]Moderate and severe categories were collapsed for analysis.
[d]Group comparisons exclude cases with missing data.
Age at death is included in all models as a linear covariate.

Table 4 HS-Ageing versus presence of regional arteriolosclerosis in UK-ADC participants

Area	HS-Ageing *n*, % of group	*n*	No HS-Ageing *n*, % of group	*n*	*P*	Significant
Frontal cortex (BA 9)	17 (68.0)	25	36 (25.0)	144	0.0001	Yes
Temporal cortex (BA 21/22)	14 (56.0)	25	35 (24.3)	144	0.0032	No
Parietal cortex (BA 39/40)	12 (52.2)	23	38 (26.0)	146	0.022	No
Occipital cortex (BA 17/18)	14 (60.9)	23	36 (25.2)	143	0.0018	Yes
Amygdala	14 (60.9)	23	29 (19.9)	146	0.0002	Yes
Entorhinal cortex (BA 28)	12 (52.2)	23	18 (12.7)	142	<0.0001	Yes
Hippocampus CA1	8 (34.8)	23	18 (12.5)	144	0.0094	No
Subiculum	3 (13.0)	23	14 (9.7)	144	0.59	No
Posterior cingulate (BA 23)	14 (66.7)	21	21 (25.3)	83	0.0014	Yes
Anterior cingulate (BA 24)	15 (65.2)	23	26 (26.0)	100	0.0016	Yes
Thalamus	13 (56.5)	23	28 (21.4)	131	0.0023	Yes
Caudate	15 (65.2)	23	23 (16.7)	138	<0.0001	Yes
Putamen	15 (65.2)	23	25 (17.9)	140	<0.0001	Yes
Insular cortex (BA 13)	14 (60.9)	23	28 (20.7)	135	0.0005	Yes
Internal capsule	4 (17.4)	23	9 (6.5)	138	0.0767	No
Globus pallidus	14 (63.6)	22	17 (63.6)	135	<0.0001	Yes

BA = Brodmann area; *P*-value determined by logistic regression controlling for age at death by covariate adjustment; the Bonferroni-Holm method was used to correct for multiple comparisons.

Table 5 Digital pathologic measurements in grey matter and white matter using antibodies against SMA and CD34

	SMA-GM*		SMA-WM*		CD34-GM*		CD34-WM*		Ratio**: HS/non-HS			
Parameter	HS	non-HS	HS	non-HS	HS	non-HS	HS	non-HS	SMA-GM	SMA-WM	CD34-GM	CD34-WM
Number of vessels	409.2	423.8	347.5	349.7	7751.2	7920.7	2715.6	2717.3	0.966	0.994	0.979	0.999
Total analysis area (μm^2)/10^7	4.03	3.97	4.02	3.69	4.02	3.92	3.96	3.68	1.015	1.091	1.026	1.075
Average stain intensity	101.4	101.0	104.2	103.3	140.7	141.1	135.0	135.5	1.004	1.009	0.997	0.997
Microvessel density # /(μm^2)*10^6	1.02	1.07	0.86	0.96	19.27	20.23	6.87	7.57	0.952	0.898	0.952	0.907
Mean vessel area (μm^2)	352.5	324.7	327.3	315.6	123.1	120.8	201.5	195.1	1.086	1.037	1.019	1.033
Median vessel area (μm^2)	151.3	141.5	137.4	124.7	72.1	72.3	115.4	110.9	1.069	1.102	0.997	1.041
Mean vessel perimeter (μm)	122.3	116.6	140.3	133.9	61.9	60.7	82.3	81.0	1.049	1.048	1.021	1.016
Median vessel perimeter (μm)	79.1	75.3	79.0	74.3	43.2	43.2	55.1	54.3	1.051	1.063	1.000	1.014
Mean vascular area (μm^2)	280.8	256.3	263.7	250.5	118.1	115.6	193.8	187.4	1.096	1.053	1.022	1.034
Median vascular area (μm^2)	113.5	104.6	102.9	94.1	71.7	71.8	113.9	109.7	1.085	1.093	0.999	1.038
Mean vessel wall thickness (μm)	1.817	1.731	1.471	1.480	1.694	1.694	2.069	2.024	1.050	0.994	1.000	1.022
Median vessel wall thickness (μm)	1.405	1.353	1.211	1.233	1.631	1.635	2.023	1.983	1.038	0.982	0.998	1.020

For comparisons, only hippocampal sclerosis versus non- hippocampal sclerosis comparisons were tested (Student's one-tailed *t*-tests).
GM = grey matter; WM = white matter; HS = hippocampal sclerosis
Black highlight *$P < 0.05$
**For ratios <1, 1, >1

noted in cases with HS-Ageing versus controls. This may indicate a technical artefact because neuropathologists may be reluctant to note arteriolosclerosis in an area already determined to show hippocampal sclerosis. The positive link between HS-Ageing pathology and arteriolosclerosis in other parts of the brain provided the bases for further analyses as shown below.

Hippocampal sclerosis of ageing pathology and small blood vessel morphology: digital pathology

To further explore the strong link between HS-Ageing and arteriolosclerosis suggested by multiple large data sets, digital microvessel analyses were performed on sections of frontal cortex (Brodmann area 9); the goal was to identify characteristics that would separate cases with and without HS-Ageing pathology. The cases used for digital pathology incorporated a representative spectrum of Alzheimer's disease pathology, arteriolosclerosis severity, hypertension, diabetes, and apolipoprotein E (*APOE*) alleles, with and without HS-Ageing pathology (Supplementary Table 3). Representative results of immunostaining blood vessels in human neocortex are shown in Fig. 3 (lower-power photomicrographs are provided in Supplementary Fig. 2), and our process for deciding which immunostains to use in our digital pathological analyses is shown in Fig. 4.

From the convenience sample ($n = 57$ different cases) used for detailed digital pathological analyses, which included 15 cases with

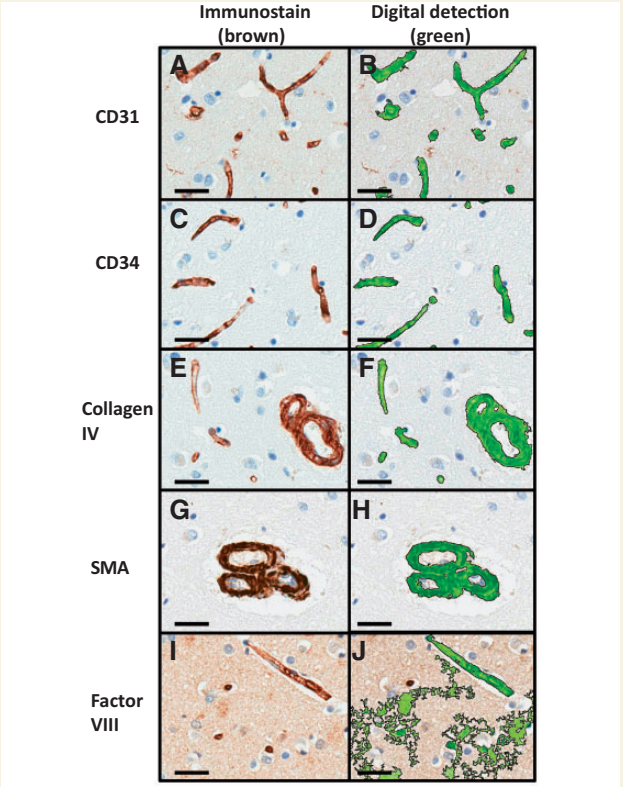

Figure 3 Vascular immunohistochemistry with multiple antibody markers, including CD 31 (**A** and **B**), CD 34 (**C** and **D**), collagen IV (**E** and **F**), α-SMA (**G** and **H**) and factor VIII (**I** and **J**). The pictures on the right denote the mark-up generated by the microvessel analysis algorithms, tailored to each individual stain. Actual immunohistochemical stain is brown (**A**, **C**, **E**, **G** and **I**) whereas the digital detection of those markers is false-coloured green (**B**, **D**, **F**, **H** and **J**) after digital analyses were run. This is the basis for further *in silico* analyses of the blood vessel morphology. Scale bars = 25 mm.

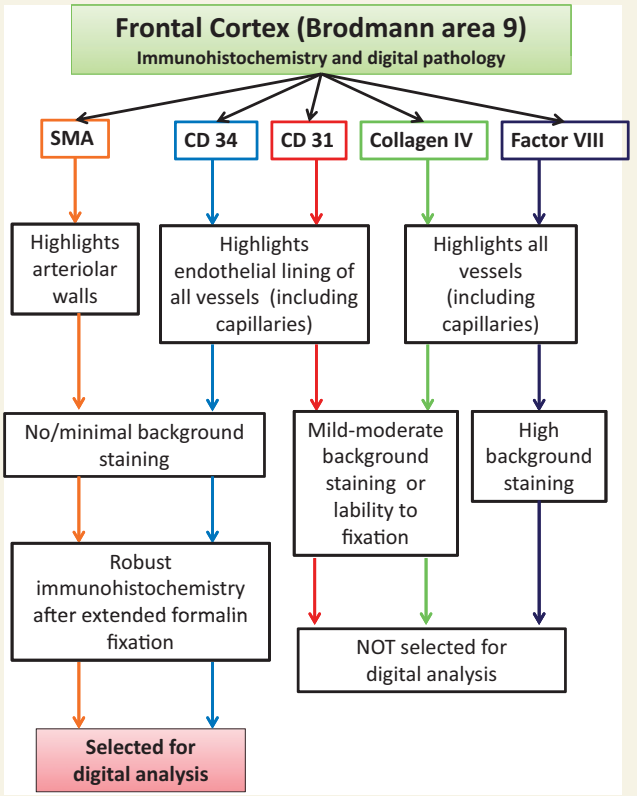

Figure 4 Process for characterization and optimization of immunohistochemistry and digital pathology. The flowchart shows immunohistochemical markers that were assessed initially to optimize technical properties suitable for digital pathological analyses of small blood vessels, along with the criteria that were applied to choose the markers to be included in, or excluded from, additional digital pathological studies.

HS-Ageing pathology, 43 834 α-SMA+ vascular profiles and 603 798 CD34+ vascular profiles were evaluated. Within the grey matter, cases with HS-Ageing showed larger mean vessel areas (352.5 μm^2 versus 324.7 μm^2), mean vessel perimeters (79.1 μm versus 75.3 μm), and mean vascular areas (280.8 μm^2 versus 256.3 μm^2) than those cases without HS-type pathology by α-SMA immunostaining (Table 6; $P < 0.05$ for each of these comparisons using one-tailed Student's *t*-test). In addition, cases with HS-Ageing showed significantly thicker vessel walls (mean thickness 1.817 μm versus 1.731 μm) within the grey matter. Figure 5 shows data derived from analyses of grey matter, stratifying on HS-Ageing and Alzheimer's disease pathologies. While not necessarily reaching statistical significance with the other variables, cases with HS-Ageing pathology show a trend for higher values in nearly all the other vascular size parameters in the larger vessels identified by α-SMA, illustrated by the ratio of HS-Ageing/non-HS-Ageing cases shown (Table 5).

Differences in blood vessel morphology between cases and controls were less apparent in white matter than grey matter. In the white matter, only the median α-SMA vessel perimeter remained significantly larger in the cases with HS-Ageing pathology (79.0 μm versus 74.3 μm) (Fig. 6 and Table 6). The changes linked to HS-Ageing pathology were at least somewhat disease-specific since there was a lack of group-level differences in these parameters according to Alzheimer's disease pathological status. Cases with Alzheimer's disease tended to have slightly higher density of grey matter α-SMA-immunoreactive blood vessels in comparison with non-Alzheimer's disease cases (data not shown). However, the Alzheimer's disease pathology-based comparisons indicate that the blood vessel perimeter changes in cases with HS-Ageing pathology are not merely a non-specific change related to neurodegeneration.

No significant differences were noted with the CD34-immunopositive vascular profiles stratifying by HS-Ageing pathological status; the blood vessel morphological changes in cases with HS-Ageing are preferentially within the α-SMA-immunoreactive arterioles. The observation of thicker arterioles in the frontal cortex of cases with HS-Ageing pathology, but not Alzheimer's disease pathology, is compatible with the semi-quantitative data analysed from large autopsy series above, indicating that arteriolosclerosis is linked to HS-Ageing pathology (Fig. 7).

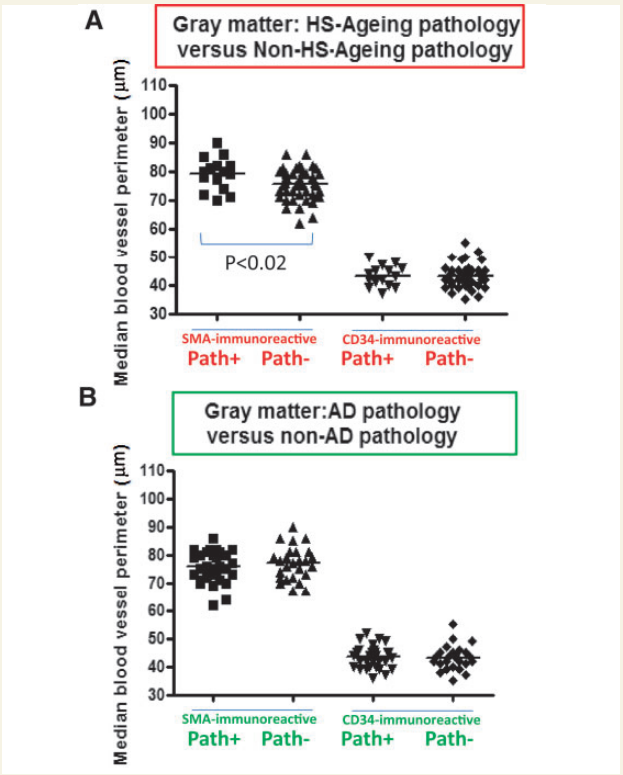

Figure 5 Grey matter (frontal cortex, Brodmann area 9): median blood vessel profile perimeter stratified according to neuropathological findings and by immunohistochemical stain used to visualize the blood vessels. Each data point represents staining of a single case in the grey matter. Note that anti-α-SMA antibody visualizes larger blood vessel profiles (arterioles) in comparison with anti-CD34 (capillaries). (**A**) Cases with HS-Ageing pathology ($n = 16$) were compared to cases without HS-Ageing pathology ($n = 51$). The cases with HS-Ageing pathology have larger median blood vessel profiles when visualized with anti-α-SMA ($P < 0.03$ using one-tailed Student's t-test) but not with anti-CD34. (**B**) Cases stratified by Alzheimer's disease pathology (cases with moderately or severely dense neuritic amyloid plaques, $n = 31$, versus cases with none or mild neuritic plaques, $n = 26$) show no difference in median blood vessel perimeter.

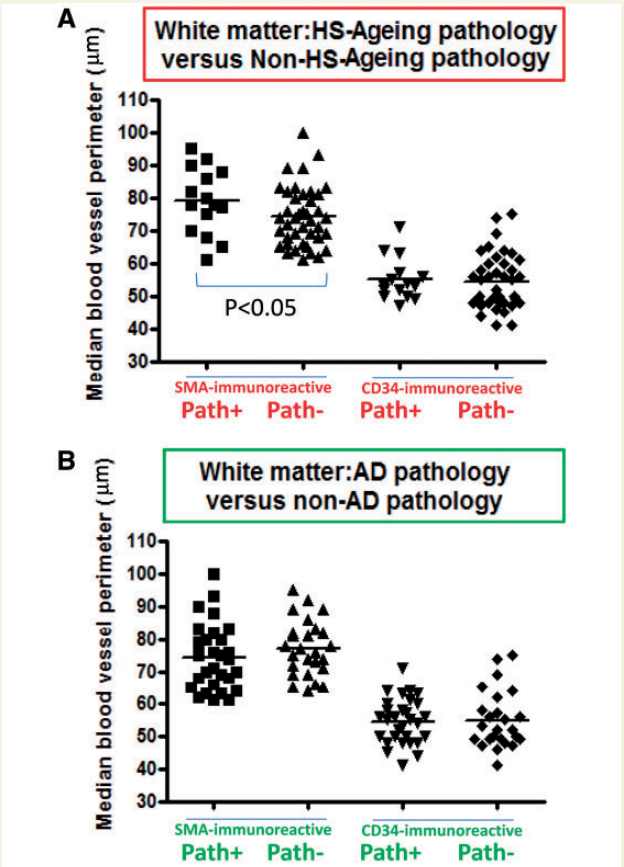

Figure 6 White matter (frontal cortex, Brodmann area 9): median blood vessel profile perimeter stratified according to neuropathological findings and by immunohistochemical stain used to visualize the blood vessels. Each data point represents staining of a single case in the white matter. (**A**) Cases with HS-Ageing pathology ($n = 16$) were compared to cases without HS-Ageing pathology ($n = 51$). The cases with HS-Ageing pathology have larger median blood vessel profiles when visualized with anti-α-SMA ($P < 0.05$ using one-tailed Student's t-test) but not with anti-CD34. (**B**) Cases stratified by Alzheimer's disease pathology (cases with moderate or severe density of neuritic amyloid plaques, $n = 31$, versus cases with none or mild neuritic plaques, $n = 26$) show no difference in median blood vessel perimeter. Note that the changes in grey matter and white matter (compare Figs 5 and 6) are roughly similar.

Discussion

Data from multiple large autopsy series were analysed to study cerebrovascular pathological parameters in patients with HS-Ageing pathology. These studies incorporated >200 subjects with autopsy-proven HS-Ageing pathology and ~2000 controls. We found no definitive link between cardiac risk factors (including hypertension and diabetes) and HS-Ageing pathology. Brains with HS-Ageing pathology also tend to have arteriolosclerosis in multiple cortical and subcortical regions. We report for the first time digital pathological quantification of geometric properties of small human brain blood vessels using CD34 and α-SMA immunohistochemistry, coupled with digital scanning and software-based image recognition, which increased the quantitative rigor of our study. These observations and analyses indicate that there is a relatively specific association between HS-Ageing and arteriolosclerosis pathologies.

There are potential limitations inherent to these analyses. The retrospective assessment of pathological data from large autopsy series entails potential sources of bias despite the large sample sizes and rigorous pathological assessments. For example, recent years have seen an increase in recognition of the impact and importance of HS-Ageing, so neuropathologists' tendency to arrive at that diagnosis may have increased. We also note that the existing definitions used to delineate the severity of arteriolosclerosis (described in the 'Materials and methods' section above) are vague, and thus may vary according to many factors. However, we do not know of a specific source of systematic bias that would

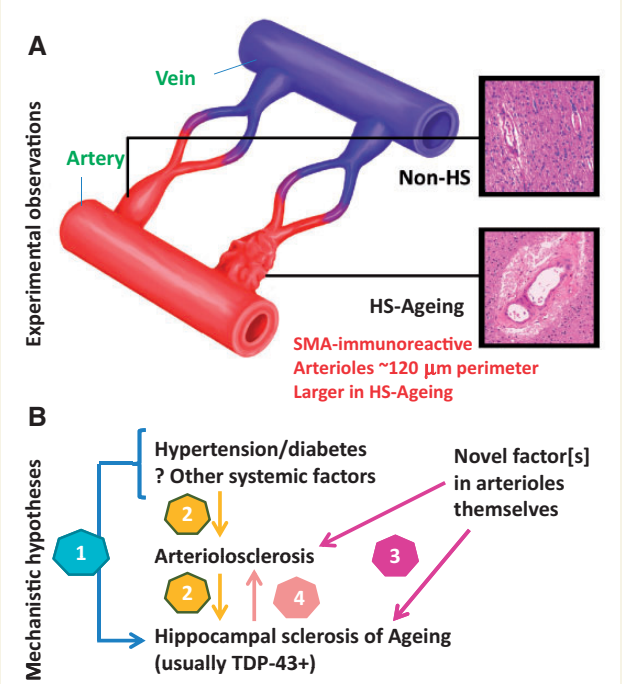

Figure 7 Model of the potential relationships between arteriolosclerosis and HS-Ageing. (**A**) The current study indicates a strong association between arteriolosclerosis and HS-Ageing pathology in elderly persons' brains. Digital pathology strongly underscores the relevance of arterioles themselves. (**B**) These observations are compatible with at least four separate mechanistic hypotheses. First, systemic factors such as hypertension independently may contribute to HS-Ageing (blue arrow) and if this were true the correlation with arteriolosclerosis may be coincidental. We found no evidence for this hypothesis. Second, unknown systemic factors may cause arteriolosclerosis which independently itself contributes to HS-Ageing pathology (yellow arrows). Third, as-yet uncharacterized factors in small blood vessels could more directly contribute to and/or manifest as both arteriolosclerosis and HS-Ageing (dark pink arrows). Fourth, HS-Ageing pathology may promote arteriolosclerosis (light pink arrows). Future studies are required to test these hypotheses.

cause the HS-Ageing and arteriolosclerosis pathologies to be spuriously correlated with each other. Note that for two of the autopsy series used in the current study (UK-ADC and the Nun Study, the latter of which used cases that had been evaluated before 2010), all of the pathological analyses were performed at the same site, the University of Kentucky. Another issue with regard to the retrospective analyses of autopsy series is missing data, which is a concern even in cohorts such as the UK-ADC where clinical data are relatively complete. However, it is not obvious how the correlation between HS-Ageing pathology and arteriolosclerosis would be because of missing data alone. A final caveat is that there are not universally applied rubrics for pathological diagnoses of either HS-Ageing or arteriolosclerosis. A recent study (Rauramaa *et al.*, 2013) described guidelines for pathological description of hippocampal pathology including hippocampal sclerosis but most of these patients were younger than 80 at death. The lack of standardized and universally applied diagnostic rules for HS-Ageing is directly relevant to the current study because only about half of cases noted to have HS-Ageing pathology were also found to have arteriolosclerosis pathology. As neuropathologists are not necessarily sensitized to low-severity arteriolosclerosis, a higher actual proportion of cases with HS-Ageing may have arteriolosclerosis than the current analyses would indicate. We also note that among other strong, proven risk factors for neurodegenerative diseases, APOE accounts for less than half of cases with Alzheimer's disease, and progranulin (GRN) mutations cause less than half of FTLD cases.

Previous studies have provided support for the hypothesis that HS-Ageing pathological changes in the hippocampus are just one component of a brain-wide pathological process (Dickson *et al.*, 1994; Nelson *et al.*, 2011; Zarow *et al.*, 2012). Of most relevance to the current study, Dickson and colleagues (1994) noted in a seminal autopsy study that 12 of 13 evaluated cases with hippocampal sclerosis demonstrated pathologically-confirmed arteriolosclerosis (Dickson *et al.*, 1994). Using multiple data sets and digital pathology, we here confirm the strong association between arteriolosclerosis and HS-Ageing pathologies. We also added new observations: the association is surprisingly specific to arteriolosclerosis and is neither associated with other cerebrovascular pathology subtypes nor with known clinical cardiovascular risk factors. Furthermore, the arteriolosclerosis changes localize to α-SMA-immunoreactive arterioles rather than CD34-immunoreactive capillaries, and particularly grey matter vessels, and these changes were not seen in Alzheimer's disease brains.

The strong association between arteriolosclerosis and HS-Ageing pathologies provides the basis for hypotheses about HS-Ageing pathogenesis (Fig. 7) and also raises at least three key questions. First, what else, besides HS-Ageing, is associated pathogenetically with arteriolosclerosis? Second, is there a specific link between arteriolosclerosis and the previously described molecular pathological correlate of HS-Ageing, namely aberrant TDP-43 immunoreactivity? And finally, why don't other subtypes of cerebrovascular pathology correlate with HS-Ageing?

Much is still unknown about both arteriolosclerosis and HS-Ageing pathologies. There is a widely recognized link between hypertension and arteriolosclerosis in the brain and other organs (Dozono *et al.*, 1991; Venkatachalam *et al.*, 2010; Kanbay *et al.*, 2011). However, we found that the strength of the association between hypertension and arteriolosclerosis is considerably weaker than the association between arteriolosclerosis and HS-Ageing pathology. This may be partly due to imperfect pre-mortem data. Future studies may discover that there is indeed a stronger link between hypertension or other systemic factors (for example, diabetes) and HS-Ageing pathology than we could find evidence for in these cohorts. A contrasting hypothesis is that constitutional factors in the blood vessel walls themselves are directly linked to arteriolosclerosis and thus to HS-Ageing. There have previously been observations of aged humans' cortical blood vessels, including vessel wall 'tortuosity' and collagenosis (Brown *et al.*, 2002, 2009; Farkas *et al.*, 2006; Thore *et al.*, 2007). We note that some previous studies of arteriolar tortuosity focused on white matter blood vessels, using thicker sections, whereas our digital pathological methods find arteriolar changes in both grey and white matter (Thore *et al.*, 2007; Brown *et al.*, 2009; Brown and

Thore, 2011). We did not perform a comprehensive study of blood vessel morphology related to Alzheimer's disease pathology, as previous studies have focused on that area (Thal *et al.*, 2003; Bouras *et al.*, 2006; Hunter *et al.*, 2012). As the cases with mild-moderate arteriolosclerosis seem to be at increased risk of HS-Ageing in the larger NACC sample, this may indicate a specific risk factor that is different from what causes severe arteriolosclerosis. It is difficult to assess the physiological impact of the blood vessel changes we observe in terms of haemodynamics or metabolic impact. Unfortunately, there is still incomplete comprehension of how arteriolosclerosis impacts the brain or how the cells of the blood vessel wall contribute to arteriolosclerosis vulnerability.

If arteriolosclerosis is pathogenetically linked with HS-Ageing, then there may also be a connection with TDP-43 molecular pathology because the large majority of cases with HS-Ageing show aberrant TDP-43 immunoreactivity (Amador-Ortiz *et al*., 2007; Nelson *et al*., 2011). By contrast, neither acute hypoxia nor epilepsy-linked causes of hippocampal injury induce TDP-43 pathology (Lee *et al*., 2008; Nelson *et al*., 2011) despite causing a pathological picture that may also be referred to as 'hippocampal sclerosis'. We previously described the lack of correlation between large-vessel cerebrovascular pathology and HS-Ageing, and described how it is important to perform the correlation mindful of the fact that cerebrovascular pathology and HS-Ageing are both highly prevalent in older individuals' brains (Nelson *et al*., 2011). Together these observations indicate that HS-Ageing pathology is not simply a response to ischaemic-reperfusion injury to the brain. Aberrant TDP-43 immunoreactivity also is seen in FTLD in association with mutations in *GRN* (Baker *et al.*, 2006; Ahmed *et al.*, 2007), in kindreds bearing mutations in some other FTLD- and amyotrophic lateral sclerosis-linked genes (Van Langenhove *et al.*, 2012), and in some cases of chronic traumatic encephalopathy (McKee *et al.*, 2010; Saing *et al.*, 2011). As the gene product of *GRN*, the progranulin protein, is thought to play a physiological role in wound repair and inflammation (Eriksen and Mackenzie, 2008), there might be a mechanistic link between chronic mechanical injury and TDP-43 pathology, with arteriolosclerosis contributing to (or manifesting in parallel with the neuronal pathology as a result of common upstream stimuli causing) TDP-43 pathology.

From a technical standpoint, the digital pathology image analysis evaluation provided a key added dimension to our study because we could analyse multiple geometric properties of many thousands of small blood vessels in a relatively unbiased manner. This method offers more quantitative and robust data than most previous methods for small blood vessel analyses. Rather than only finding that 'arteriolosclerosis' is increased in HS-Ageing, we could reach more biologically-informative conclusions: α-SMA-immunoreactive small blood vessels (presumably comprising mostly arterioles) have thicker walls, larger perimeter, and larger lumen size, whereas smaller CD-34 immunoreactive profiles (mostly capillaries) are not significantly altered in HS-Ageing versus controls. These observations fit with previous work focusing on digital pathological analyses of Alzheimer's disease neuropathology (Armstrong and Cairns, 2009; Murray *et al.*, 2011; Robinson *et al.*, 2011; Neltner *et al*., 2012), and indicate that digital pathological evaluations do indeed provide an added dimension for neuropathological investigations, enabling us to surmount some of the human eye's limitations.

Overall, arteriolosclerosis is positively correlated with HS-Ageing pathology, and that relationship seems specific because neither other cerebrovascular pathological subtypes nor cardiovascular risk factors are strongly correlated with HS-Ageing pathology. Arteriolosclerosis was also not correlated with Alzheimer's disease pathology. The pathways and processes that cause dysfunction of brain arterioles in HS-Ageing are still unknown. These data add to an evolving appreciation that HS-Ageing is a whole-brain disease, although hippocampal neurodegeneration is currently the defining manifestation.

Acknowledgements

We are deeply grateful to all of the study participants that make this research possible. The authors thank Sonya Anderson and Ela Patel for technical support; Greg Cooper, MD, Nancy Stiles, MD and Allison Caban-Holt, PhD for the clinical evaluations; and Daron Davis, MD and Stephen W. Scheff, PhD, for pathological evaluations. Corresponding Author Peter Nelson, MD PhD had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

Research reported in this publication was supported by National Institutes of Health under award numbers P30AG028383, R01NR014189, UL1TR000117, U01 AG016976, and S10RR26489. The content is solely the responsibility of the authors and does not represent the official views of the National Institutes of Health.

Supplementary material

Supplementary material is available at *Brain* online.

References

Ahmed Z, Mackenzie IR, Hutton ML, Dickson DW. Progranulin in frontotemporal lobar degeneration and neuroinflammation. J. Neuroinflammation 2007; 4: 7.

Amador-Ortiz C, Dickson DW. Neuropathology of hippocampal sclerosis. Handb Clin Neurol 2008; 89: 569–72.

Amador-Ortiz C, Lin WL, Ahmed Z, Personett D, Davies P, Duara R, et al. TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. Ann Neurol 2007; 61: 435–45.

Amaral D, Lavenex P. Hippocampal neuroanatomy. In: Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J, editors. The hippocampus book. New York: Oxford University Press; 2007. p. 37–114.

Armstrong RA, Cairns NJ. Size frequency distribution of the beta-amyloid (abeta) deposits in dementia with Lewy bodies with associated Alzheimer's disease pathology. Neurol Sci 2009; 30: 471–7.

Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature 2006; 442: 916–9.

Beekly DL, Ramos EM, Lee WW, Deitrich WD, Jacka ME, Wu J, et al. The National Alzheimer's Coordinating Center (NACC) database: the uniform data set. Alzheimer Dis Assoc Disord 2007; 21: 249–58.

Beekly DL, Ramos EM, van Belle G, Deitrich W, Clark AD, Jacka ME, et al. The National Alzheimer's Coordinating Center (NACC) Database: an Alzheimer disease database. Alzheimer Dis Assoc Disord 2004; 18: 270–7.

Bouras C, Kovari E, Herrmann FR, Rivara CB, Bailey TL, von Gunten A, et al. Stereologic analysis of microvascular morphology in the elderly: Alzheimer disease pathology and cognitive status. J Neuropathol Exp Neurol 2006; 65: 235–44.

Brown WR, Moody DM, Challa VR, Thore CR, Anstrom JA. Venous collagenosis and arteriolar tortuosity in leukoaraiosis. J Neurol Sci 2002; 203-204: 159–63.

Brown WR, Moody DM, Thore CR, Anstrom JA, Challa VR. Microvascular changes in the white mater in dementia. J Neurol Sci 2009; 283: 28–31.

Brown WR, Thore CR. Review: cerebral microvascular pathology in ageing and neurodegeneration. Neuropathol Appl Neurobiol 2011; 37: 56–74.

Corrada MM, Berlau DJ, Kawas CH. A population-based clinicopathological study in the oldest-old: the 90+ study. Curr Alzheimer Res 2012; 9: 709–17.

Dickson DW, Davies P, Bevona C, Van Hoeven KH, Factor SM, Grober E, et al. Hippocampal sclerosis: a common pathological feature of dementia in very old (> or = 80 years of age) humans. Acta Neuropathol 1994; 88: 212–21.

Dozono K, Ishii N, Nishihara Y, Horie A. An autopsy study of the incidence of lacunes in relation to age, hypertension, and arteriosclerosis. Stroke 1991; 22: 993–6.

Eriksen JL, Mackenzie IR. Progranulin: normal function and role in neurodegeneration. J Neurochem 2008; 104: 287–97.

Farkas E, de Vos RA, Donka G, Jansen Steur EN, Mihaly A, Luiten PG. Age-related microvascular degeneration in the human cerebral periventricular white matter. Acta Neuropathol 2006; 111: 150–7.

Garde E, Mortensen EL, Krabbe K, Rostrup E, Larsson HB. Relation between age-related decline in intelligence and cerebral white-matter hyperintensities in healthy octogenarians: a longitudinal study. Lancet 2000; 356: 628–34.

Hunter JM, Kwan J, Malek-Ahmadi M, Maarouf CL, Kokjohn TA, Belden C, et al. Morphological and pathological evolution of the brain microcirculation in aging and Alzheimer's disease. PloS one 2012; 7: e36893.

Jellinger KA. Hippocampal sclerosis: a common pathological feature of dementia in very old humans. Acta Neuropathol 1994; 88: 599.

Kanbay M, Sanchez-Lozada LG, Franco M, Madero M, Solak Y, Rodriguez-Iturbe B, et al. Microvascular disease and its role in the brain and cardiovascular system: a potential role for uric acid as a cardiorenal toxin. Nephrol Dial Transplant 2011; 26: 430–7.

Kuslansky G, Verghese J, Dickson D, Katz M, Busche H, Lipton RB. Hippocampal sclerosis: cognitive consequences and contribution to dementia. Neurology 2004; 62: A128–9.

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: 305–11.

Leverenz JB, Lipton AM. Clinical aspects of hippocampal sclerosis. Handb Clin Neurol 2008; 89: 565–7.

McKee AC, Gavett BE, Stern RA, Nowinski CJ, Cantu RC, Kowall NW, et al. TDP-43 proteinopathy and motor neuron disease in chronic traumatic encephalopathy. J Neuropathol Exp Neurol 2010; 69: 918–29.

Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol 2012; 123: 1–11.

Mortimer JA. The nun study: risk factors for pathology and clinical-pathologic correlations. Curr Alzheimer Res 2012; 9: 621–7.

Murray ME, Graff-Radford NR, Ross OA, Petersen RC, Duara R, Dickson DW. Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study. Lancet Neurol 2011; 10: 785–96.

Nelson PT, Abner EL, Scheff SW, Schmitt FA, Kryscio RJ, Jicha GA, et al. Alzheimer's-type neuropathology in the precuneus is not increased relative to other areas of neocortex across a range of cognitive impairment. Neurosci Lett 2009; 450: 336–9.

Nelson PT, Abner EL, Schmitt FA, Kryscio RJ, Jicha GA, Smith CD, et al. 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 2010; 20: 66–79.

Nelson PT, Head E, Schmitt FA, Davis PR, Neltner JH, Jicha GA, et al. Alzheimer's disease is not "brain aging": neuropathological, genetic, and epidemiological human studies. Acta Neuropathol 2011a; 121: 571–87.

Nelson PT, Jicha GA, Schmitt FA, Liu H, Davis DG, Mendiondo MS, et al. Clinicopathologic correlations in a large Alzheimer disease center autopsy cohort: neuritic plaques and neurofibrillary tangles "do count" when staging disease severity. J Neuropathol Exp Neurol 2007; 66: 1136–46.

Nelson PT, Schmitt FA, Lin Y, Abner EL, Jicha GA, Patel E, et al. Hippocampal sclerosis in advanced age: clinical and pathological features. Brain 2011b; 134: 1506–18.

Nelson PT, Smith CD, Abner EL, Wilfred BJ, Wang WX, Neltner JH, et al. Hippocampal sclerosis of aging, a prevalent and high-morbidity brain disease. Acta Neuropathol 2013; 126: 161–77.

Neltner JH, Abner EL, Schmitt FA, Denison SK, Anderson S, Patel E, et al. Digital pathology and image analysis for robust high-throughput quantitative assessment of Alzheimer disease neuropathologic changes. J Neuropathol Exp Neurol 2012; 71: 1075–85.

Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006; 314: 130–3.

Polvikoski TM, van Straaten EC, Barkhof F, Sulkava R, Aronen HJ, Niinisto L, et al. Frontal lobe white matter hyperintensities and neurofibrillary pathology in the oldest old. Neurology 2010; 75: 2071–8.

Rauramaa T, Pikkarainen M, Englund E, Ince PG, Jellinger K, Paetau A, et al. Consensus recommendations on pathologic Cchanges in the hippocampus: a postmortem multicenter inter-rater Study. J Neuropathol Exp Neurol 2013; 72: 452–61.

Riley KP, Snowdon DA, Markesbery WR. Alzheimer's neurofibrillary pathology and the spectrum of cognitive function: findings from the Nun Study. Ann Neurol 2002; 51: 567–77.

Robinson JL, Geser F, Corrada MM, Berlau DJ, Arnold SE, Lee VM, et al. Neocortical and hippocampal amyloid-beta and tau measures associate with dementia in the oldest-old. Brain 2011; 134: 3708–15.

Saing T, Dick MC, Nelson PT, Kim RC, Cribbs DH, Head E. Frontal cortex neuropathology in dementia pugilistica. J Neurotrauma 2011; 29: 1054–70.

Schmitt FA, Nelson PT, Abner E, Scheff S, Jicha GA, Smith C, et al. University of Kentucky Sanders-Brown healthy brain aging volunteers: donor characteristics, procedures and neuropathology. Curr Alzheimer Res 2012; 9: 724–33.

Snowdon DA, Kemper SJ, Mortimer JA, Greiner LH, Wekstein DR, Markesbery WR. Linguistic ability in early life and cognitive function and Alzheimer's disease in late life. Findings from the Nun Study. JAMA 1996; 275: 528–32.

Thal DR, Ghebremedhin E, Orantes M, Wiestler OD. Vascular pathology in Alzheimer disease: correlation of cerebral amyloid angiopathy and arteriosclerosis/lipohyalinosis with cognitive decline. J Neuropathol Exp Neurol 2003; 62: 1287–301.

Thore CR, Anstrom JA, Moody DM, Challa VR, Marion MC, Brown WR. Morphometric analysis of arteriolar tortuosity in human cerebral white matter of preterm, young, and aged subjects. J Neuropathol Exp Neurol 2007; 66: 337–45.

Van Langenhove T, van der Zee J, Van Broeckhoven C. The molecular basis of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum. Ann Med 2012; 44: 817–28.

Venkatachalam MA, Griffin KA, Lan R, Geng H, Saikumar P, Bidani AK. Acute kidney injury: a springboard for progression in chronic kidney disease. Am J Physiol Renal Physiol 2010; 298: F1078–94.

White L, Petrovitch H, Hardman J, Nelson J, Davis DG, Ross GW, et al. Cerebrovascular pathology and dementia in autopsied Honolulu-Asia aging study participants. Ann N Y Acad Sci 2002; 977: 9–23.

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 Dis Assoc Disord 1999; 13: 226–31.

Zarow C, Sitzer TE, Chui HC. Understanding hippocampal sclerosis in the elderly: epidemiology, characterization, and diagnostic issues. Curr Neurol Neurosci Rep 2008; 8: 363–70.

Zarow C, Weiner MW, Ellis WG, Chui HC. Prevalence, laterality, and comorbidity of hippocampal sclerosis in an autopsy sample. Brain Behav 2012; 2: 435–42.