Supplementary File for:

Cerebral amyloid angiopathy distribution: a cautionary note [correct title]

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Methods Supplementary file

Tissue for the post-mortem study was obtained from cognitively impaired or cognitively normal elderly subjects recruited to longitudinal studies with post-mortem follow-up the UK. The sites from which tissue was obtained were part of the Brains for Dementia Research (BDR) network (https://bdr.alzheimersresearchuk.org/researchers/). The final tissue selection was based solely on the availability of tissue from the frontal cerebral cortex. BDR recruits from a network of 6 UK dementia research centres (King's College London, Bristol, Manchester, Oxford, Cardiff and Newcastle). Participants are predominantly older people (aged 65 years or more), 60% female and almost entirely Caucasian ethnicity (99%). Details of inclusion criteria for BDR are given in Francis et al. 2018[1]. For all brains, full neuropathological dissection, sampling, and characterization was undertaken according to a standardized BDR protocol by experienced neuropathologists in each of the 6 BDR network brain banks. This protocol, arrived at by consensus across the BDR network and based on the BrainNet Europe initiative[2], generates a narrative description of the regional pathology within the brain together with standardized scoring for Braak tangle pathology, Braak Lewy body score, Thal phase of Abeta pathology, Consortium to Establish a Registry of Alzheimer's Disease (CERAD) classification, extent, location, and classification of vascular pathology and TDP43 status.

We diluted antibodies in primary layer diluent (0.3% v/v Triton-X-100, 0.01% w/v sodium azide and 2% v/v normal serum in phosphate buffered saline) and treated with 98% formic acid for 15 minutes. The immunohistochemical determinations are of Aβ40 and Aβ42. The two antibodies used (G30 and 20G10) recognise the C-terminal neoepitopes ending at Aβ x-40 and Aβ x-42. Mouse monoclonal 20G10 was raised against the Aβ35-42 fragment and selected for its C-terminal Aβ42 specificity. Rabbit antiserum G30 was raised against Aβ35-42 for Aβ40[3]. Aβ40 and Aβ42 density was analysed as per positive vessel centimetre

45 square for each section (Figure 1). Low magnification images were captured for entire
46 sections to allow the determination of the total area (cm^2) of each section. Aβ40 and Aβ42
47 density was analysed as number of +ve vessels per cm^2. See Figure S1.

48 Mouse monoclonal 20G10 was raised against the Aβ35–42 fragment and selected for its C-
49 terminal Aβ42 specificity. Rabbit antiserum G30 was raised against CMVGGVV for Aβ40.
50 C-terminal specificity of G30 and 20G10 for Aβ40 and Aβ42, respectively, was established
51 by preincubation of the antibody with a 10-fold excess of the cognate Aβ1–40 or Aβ42
52 peptides, which completely obliterated the ability of both primary antibodies to label amyloid
53 deposits in sections of double-transgenic mouse brain. In contrast, preincubation of G30 or
54 20G10 with an excess of the other's cognate peptide had no discernible effect on
55 immunolabelling[4].

56 Sections were viewed on a Leica DMRB epifluorescence microscope (Leica Microsystems,
57 Milton Keynes, UK). Immunofluorescent labelling of Aβ42 (20G10 antibody) was viewed in
58 the green channel and Aβ40 (G30 antibody) in the red channel. All sections were viewed in a
59 blinded, random sequence. The entirety of each section was methodically examined for
60 arterial vessels containing fluorescence at 100x magnification, in both channels. Capillaries
61 were excluded. Images of positive vessels were captured for both channels with a DFC420
62 camera (Leica Microsystems). 3034 images were sampled in total. Definition of CAA
63 positivity was performed by a single blinded observer (JMRF) blind to neuropathological
64 group assignment (DRH). Examples of vessels positive for Aβ40-CAA, Aβ42-CAA, or both,
65 are shown in Supplementary Figure 1. Observer-dependent assessment of CAA positivity was
66 confirmed using automated image analysis with ImageJ software (http://rsbweb.nih.gov/ij/).
67 A threshold was defined in an unlabelled area of tissue, to eliminate background
68 fluorescence. The immunolabelled area defined by ImageJ was highly correlated with
69 observer-defined positive vessel number and was highly significant (for Aβ40, Spearman's

70 r=0.974, p<0.001). We referred to significant CAA when at leave 5 vessels or plaques were
71 Aβ40 and Aβ42 positive. Across the cohort, there were 39 cases without significant CAA (<5
72 CAA+ vessels/cm2). Among these, only 15 (38.5%) lacked significant parenchymal plaques
73 (<5 plaques/cm2), i.e., 24 (61.5%) had significant parenchymal plaques (6 Controls, 14 Other
74 neurodegenerative, 4 AD). Among the cases with significant CAA, most (23/26, 88.5%) had
75 significant parenchymal plaques. Across the cohort, the correlation between Aβ CAA+ vessel
76 density and parenchymal plaque density was low (Pearson R: 0.192 for Aβ40, 0.143 for
77 Aβ42). Within each of the 3 sub-groups, correlations were similarly low.

78 **Supplementary Figure 1.** Examples of small arterial vessels exhibiting CAA, positive for
79 Aβ40 only (top panels, A), or both Aβ40 and Aβ42 (middle panels, B) or Aβ42 only (Lower
80 panels, C). Scale bars 100 microns.

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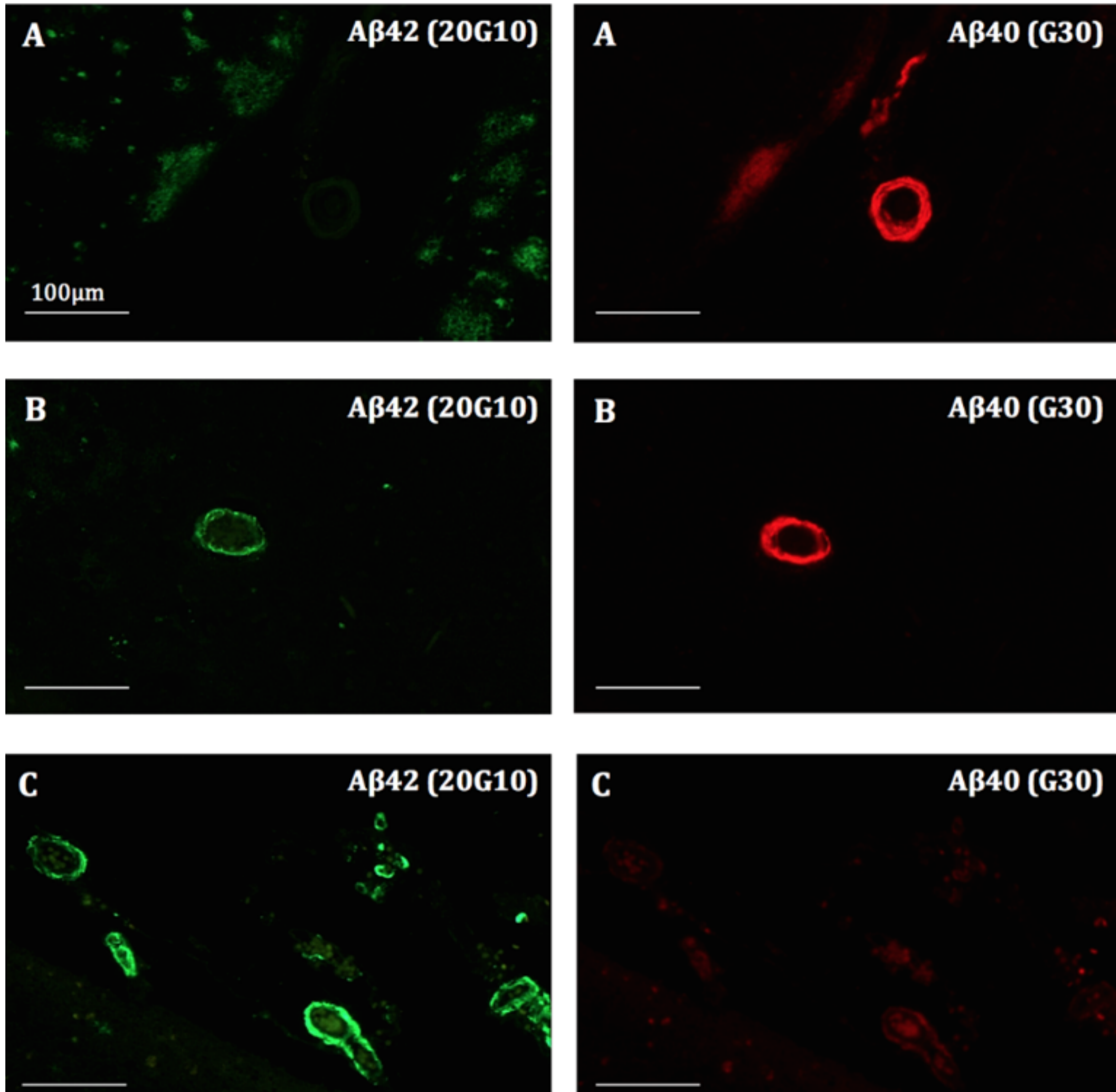

82 **Reference**

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