Original Research Article

Dementia and Geriatric Cognitive Disorders

Dement Geriatr Cogn Disord 2009;27:465–473
DOI: 10.1159/000217729

Accepted: February 23, 2009
Published online: May 7, 2009

Population Variation in Glial Fibrillary Acidic Protein Levels in Brain Ageing: Relationship to Alzheimer-Type Pathology and Dementia

S.B. Wharton[b] J.P. O'Callaghan[a] G.M. Savva[d] J.A.R. Nicoll[f] F. Matthews[e]
J.E. Simpson[b] G. Forster[b] P.J. Shaw[c] C. Brayne[d] P.G. Ince[b]
on behalf of the MRC Cognitive Function and Ageing Neuropathology Study Group

[a]Centers for Disease Control and Prevention-NIOSH, Morgantown, W.Va., USA; [b]Academic Unit of Pathology and [c]Academic Unit of Neurology, University of Sheffield, Sheffield, [d]Department of Public Health and Primary Care and [e]MRC Biostatistics Unit, Institute of Public Health, University of Cambridge, Cambridge, and [f]Division of Clinical Neurosciences, University of Southampton, Southampton, UK

Key Words
Glial fibrillary acidic protein · Gliosis · Astrogliosis · Astrocyte pathology · Alzheimer's disease · Brain ageing

Abstract
Background: The cellular pathology of astrocytes in brain ageing and their role in modulating the brain's response to neurodegenerative pathology remain incompletely understood. ***Methods:*** Using quantitative ELISA, we have investigated glial fibrillary acidic protein (GFAP) expression in the population-based neuropathology cohort of the Medical Research Council Cognitive Function and Ageing Study to determine: (1) the population variation in the astroglial hypertrophic response, (2) its relationship to the presence of Alzheimer-type pathology, and (3) its association with cognition. ***Results:*** Increasing GFAP was found with increasing Braak stage, levels increasing even at early stages. Within Braak stages, GFAP did not differ between demented and non-demented individuals, but there was greater variance in GFAP in the demented. Possession of ApoE ε4 was associated with slightly increased GFAP levels (not significant) for given amyloid β protein loads. ***Conclusion:*** In a population-based sample, increasing gliosis precedes development of Alzheimer lesions. Population variation in GFAP with varying Alzheimer lesion burdens suggests that they are not the only driver for astrogliosis. GFAP was not independently predictive of dementia, but the variation in astrocytic responses may be a factor modulating brain responses to neurodegenerative pathology.

Introduction

The most common cause of dementia in both clinic- and population-based studies is Alzheimer's disease (AD), characterised by neurofibrillary tangles (NFT), plaques and neuropil threads and at the molecular level by the deposition of amyloid β protein (Aβ) and abnormally phosphorylated tau protein. The amyloid cascade hypothesis posits that Aβ deposition is an upstream event, initiating cascades resulting in tau phosphorylation and cellular injury [1]. However, factors regulating progression of molecular and cellular pathology between individuals remain to be defined. Whilst NFT and neuritic plaques correlate with dementia and are the key diagnostic markers for AD, population-based neuropathology approaches,

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1420–8008/09/0275–0465$26.00/0

Accessible online at:
www.karger.com/dem

S.B. Wharton
Academic Unit of Pathology, University of Sheffield Medical School
Beech Hill Road
Sheffield, S10 2RX (UK)
Tel. +44 114 226 1342, Fax +44 114 226 1464, E-Mail s.wharton@sheffield.ac.uk

such as the Medical Research Council (MRC) Cognitive Function and Ageing Study (CFAS), have shown considerable overlap in lesion burden between demented and non-demented individuals [2], so that burdens of classical AD-type pathology alone are incomplete predictors of cognitive status. There is a need to identify factors that affect lesion progression and the brain's tolerance to loads of Alzheimer-type pathology, or that have independent effects on cognitive function. Such factors may contribute to interindividual variation in cognitive outcomes for given loads of Alzheimer-type pathology and associated mixed pathologies, whilst the consideration of non-classical pathological markers may improve the explanatory power of pathological models for dementia.

Astrocytes (and microglia) have potentially important roles in brain ageing and age-related neurodegeneration [3]. Astrocytes react to Alzheimer-type pathology [4], colocalising to senile plaques, and may be important in plaque progression [5] and clearance [6]. Aβ peptide, particularly in aggregated form, can activate glia [7] and is toxic to astrocytes [8]. However, other factors may also lead to changes in astrocyte phenotype, including age-related oxidative stress and activation of inflammatory mechanisms, so that glial activation may be an important contributor to the development of Alzheimer-type pathology, and by extension cognitive impairment, rather than just a consequence [9]. Activated astrocytes may modulate the effects of Alzheimer-type pathology on the brain. Factors involved may include cytokine production and clearance of Aβ peptide [10]. Astrocytes also contribute to maintenance of the extracellular milieu, metabolic needs of neurons and neuronal communication through active participation at the synapse [11]. So, loss of astrocytic function, through damage or altered phenotype, may contribute to the effects of Alzheimer-type pathology on cellular processes underlying cognition. Therefore astrocytic responses may be a factor in the resilience of individual brains to the effects of brain ageing and Alzheimer-type pathology on cognition.

The nature and variation of the astrocyte response and its cellular pathology remains to be fully defined in the ageing brain. Astrogliosis is a hypertrophic response that can be demonstrated in tissue sections by immunohistochemistry for the intermediate filament protein glial fibrillary acidic protein (GFAP), but which also involves upregulation of the other intermediate filament proteins vimentin, nestin and synemin [12, 13]. In animal models, GFAP expression increases with age and may be driven by oxidative stress [14]. In case-control studies, GFAP is elevated in AD, increasing with tangle burden and disease duration [15]. GFAP expression also increases with ageing in the human and there is evidence that it correlates inversely with cognitive function, independently of Alzheimer-type pathology [16].

We, and others, have shown that gliosis is common in the neocortex in brain ageing in a population-based sample derived from the MRC CFAS neuropathology study [17]. As a population-based cohort, this allows unbiased study of interindividual variation of pathology, its relationship to cognitive impairment and potential risk factors across the whole spectrum of ageing in a sufficiently powered sample [2, 18]. Using conventional immunohistochemistry in the temporal neocortex, we showed wide population variation in gliosis, from very little, largely confined to layer 1, through patchy to confluent patterns of immunoreactivity. We also showed a relationship to the burden of Alzheimer-type pathology, particularly the presence of neuritic plaques. However, gliosis was also common in those with very low burdens of Alzheimer-type pathology, with wide overlaps between individuals with different Braak stages of pathology [19], and poor correlations with both local and global brain measures of Alzheimer-type pathology.

Immunohistochemistry for GFAP is a well-established method to assess gliosis. However, although the extent of staining can be quantified, measures obtained cannot be considered to truly represent concentrations of GFAP in a linear fashion [20]. Thus, relationships of gliosis to Alzheimer-type pathology and to cognitive impairment may be obscured. Therefore, in this paper, we have sought to refine our estimates of the population variation in gliosis in the ageing temporal cortex using an ELISA-based method, which is more quantitative and provides complementary information to immunohistochemistry. This technique has previously demonstrated higher levels of GFAP in AD cases than controls, with the most prominent glial response in temporal lobe [21].

We therefore investigated the population variation in GFAP expression using a quantitative ELISA method, its relationship to progressively increasing burdens of Alzheimer-type pathology and whether GFAP levels may explain some of the variation in cognitive outcomes in ageing, independently of Alzheimer-type pathology. We hypothesised that, for given loads of Alzheimer-type pathology, levels of GFAP expression would be higher in demented than in non-demented individuals. We also hypothesised that *APOE* genotype affects the astrocyte hypertrophic response, specifically that the ε4 genotype would be associated with a greater astrocyte response for given loads of Alzheimer-type pathology.

Methods

Tissue and Cohort

This study used all of the cases derived from one of the centres (Cambridge) of the MRC CFAS neuropathology cohort. The use of all cases from one centre was designed to maintain the unbiased, population-based study design. The CFAS cohort has been described in detail elsewhere [2, 17, 18]. Briefly, baseline prevalence screening of the cohort included sociodemographic, cognitive, functional health and medication variables. Detailed assessment waves in 20% included a standardised assessment for psychiatric disorders in older people (GMS AGECAT). This 20% sample was weighted towards impaired respondents but also included non-demented respondents. All those who took part in assessment interviews were approached to ask whether they and their families were willing to consider brain donation after the respondent's death. Dementia status at death was based on review of all information available from the respondent and informants during the last years of life, an informant interview after death and death certification. The cohort from the centre used in this study included 96 cases. Multicentre research ethics committee approval was obtained for this study.

Neuropathology had previously been assessed by neuropathologists, blinded to all clinical data, using a modified CERAD protocol (www.cfas.ac.uk), which semi-quantifies diffuse plaques, neuritic plaques and NFT respectively as none, mild, moderate or severe [22]. Cases were also staged with the Braak staging scheme of NFT, detected by immunostaining with the AT8 antibody to phosphorylated tau protein [23].

Immunohistochemistry for GFAP and Aβ

This study was carried out in the lateral temporal cortex. Immunohistochemistry and image analysis of GFAP and Aβ have been described previously [17]. Briefly, immunohistochemistry was performed on sections cut from paraffin-embedded formalin-fixed blocks of temporal cortex. Immunohistochemistry for GFAP was carried out using a standard ABC method with a rabbit IgG antibody (Dako Cytomation, Ely, UK) at 1:1,000 dilution, incubated for 1 h at room temperature. Antigen retrieval was carried out using microwaving for 10 min in trisodium citrate buffer. The antibody to Aβ (Dako Cytomation) was used at 1:100, incubated overnight at 4°C, following antigen retrieval. Image capture for analysis of area staining was performed using CellR software (Olympus Biosystems, Hamburg, Germany).

ELISA for GFAP

Frozen tissue from the contralateral lateral temporal cortex was used for protein extraction for the ELISA analysis. ELISA GFAP data were obtained from 76 cases. Brain slices from this cohort of CFAS had been frozen using liquid nitrogen, then stored at –80°C. A portion of temporal cortex was subdissected from the relevant frozen brain slice. Samples were prepared for assay by sonication in hot (approx. 90°C) 1% SDS. GFAP was assayed in accordance with a previously described ELISA for which detailed protocols have been provided [24, 25]. In brief, a rabbit polyclonal antibody to GFAP was coated on the wells of Immulon-2 microtitre plates (Thermo Labsystems, Franklin, Mass., USA). The detergent-denatured homogenates and standards were diluted in phosphate-buffered saline (pH 7.4) containing 0.5% Triton-X 100 solution. Non-specific binding was blocked by the addition of 5% non-fat dry milk and aliquots of the homogenate and standards were added to the wells and incubated at 37°C. Following washes, a mouse monoclonal antibody to GFAP was added to 'sandwich' the GFAP between the two antibodies. An alkaline phosphatase-conjugated antibody directed against mouse IgG was then added and a coloured reaction product was obtained by subsequent addition of the enzyme substrate, *p*-nitrophenol. Quantification was achieved by spectrophotometry of the coloured reaction product at 405 nm in a microplate reader, Spectra Max Plus, and analyzed with Soft Max Pro Plus software (Molecular Devices, Sunnyvale, Calif., USA). GFAP concentration is expressed in micrograms of GFAP/milligram total protein.

APOE *Genotyping*

APOE genotyping was performed as previously described [26], but using samples of frozen brain tissue. Briefly, tissue samples were digested with proteinase K and heated to 95°C followed by PCR amplification of the polymorphic fragment of the gene using established primers [27]. The PCR products were digested with the restriction enzyme *Hha* I. The resulting fragments were separated according to size by polyacrylamide gel electrophoresis, visualised and photographed.

Statistical Analysis

Statistical analysis was performed using SPSS version 14.0. Trends across groups were tested using the Jonckheere-Terpstra test. Correlations were performed using Spearman's rank test (two-tailed). The relationship between ApoE genotype, Aβ and GFAP was tested using linear regression with log-transformed GFAP and logistic-transformed Aβ levels. Linear regression using log-transformed GFAP was used to test whether any observed relationships between pathological lesions and GFAP were mediated by age.

Results

Population Variation in GFAP

GFAP protein expression by ELISA showed wide population variation (mean = 8.4 μg GFAP/mg total protein, standard deviation = 5.1; median = 7.3, interquartile range = 5.8). There was only a weak positive relationship between ELISA GFAP and GFAP area expression as measured by immunohistochemistry ($p = 0.07$, $r_s = 0.209$) (fig. 1).

Relationship of GFAP to Alzheimer-Type Pathology

We examined the relationship of ELISA GFAP to Braak stage as a global brain measure of Alzheimer-type pathology. ELISA GFAP increased with increasing Braak group ($p < 0.001$). Notably, levels of GFAP varied in all Braak groups and the levels were noted to rise even in association with limbic stage NFT (Braak stages III–IV) (table 1). We further examined whether ELISA GFAP expression increased in relation to local measures of Alz-

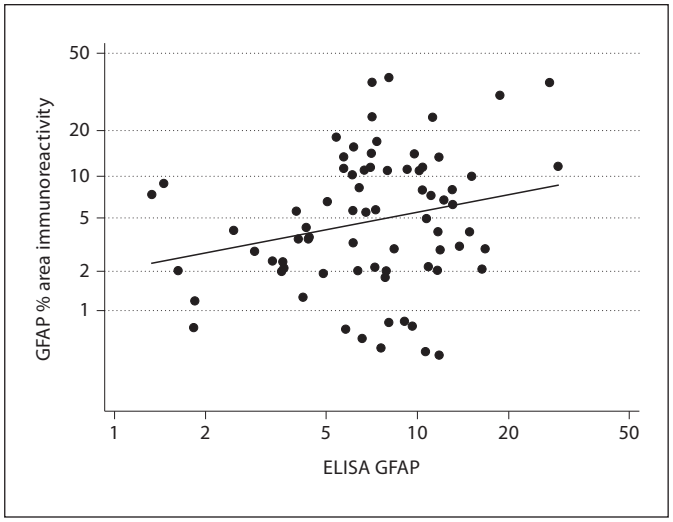

Fig. 1. Scatter plot of percent area immunoreactivity of GFAP versus (contralateral) ELISA-measured GFAP.

heimer-type pathology in the (contralateral) temporal cortex. Moderate and severe groups were amalgamated because of low numbers in the severe group. ELISA GFAP increased with increasing neuritic plaque score ($p = 0.003$). The difference was less marked for diffuse plaques ($p = 0.03$). ELISA GFAP also showed a significant trend to increase with NFT score ($p = 0.003$) (fig. 2). The relationships between GFAP and other pathological lesions were attenuated by around 25%, but remained statistically significant, after adjusting for age (data available on request).

The effects of co-existing vascular pathology on levels of GFAP were considered (data not shown). There were no differences in levels of GFAP in those with no brain vascular disease as compared to those with vascular disease as assessed by either the presence of a single vascular lesion (infarct, haemorrhage or lacune), small-vessel disease only, or multiple vascular pathologies. Haematoxylin-and-eosin-stained sections from the paraffin blocks of the temporal cortex were also assessed for local vascular pathology. These sections were from the same blocks as were used for GFAP immunostaining and contralateral to the temporal cortex used for GFAP ELISA. Only 6 cases showed either infarcts affecting part of the tissue, or microinfarcts. These cases did not show elevated GFAP, either by area immunostaining or ELISA, compared to those without infarcts. Vascular pathology did therefore not account for cases with elevated GFAP.

Variation in GFAP Protein Levels by Dementia Status and Braak Stage

We investigated whether GFAP levels varied between demented and non-demented individuals for given loads of Alzheimer-type pathology. Braak stage for NFT was used as a global brain assessment of Alzheimer-type pathology. This defines 6 anatomical stages of tangle progression and shows a correlation with cognitive status [19]. We combined stages 0–II, III–IV and V–VI to represent entorhinal, limbic and isocortical stages of spread. Isocortical NFT are characteristic of this latter stage. In both entorhinal (stages 0–II) and limbic (III–IV) stages, the mean GFAP level did not differ significantly between the demented and non-demented. However, in both cases the variance in GFAP expression was higher in the demented group (entorhinal stage $p = 0.031$, limbic stage $p = 0.026$), including cases with both lower and higher levels of GFAP than in the non-demented group. There was only one individual without dementia in the isocortical stage (fig. 3; table 1). Similar results were obtained when other measures of Alzheimer-type pathology (plaque and tangle scores) were used. GFAP increased with age, but there was no evidence that GFAP affects dementia at any age.

Variation in the Relationship between Aβ and GFAP by APOE *Genotype*

We investigated whether GFAP expression by ELISA was higher for given levels of Aβ (as determined by immunohistochemistry) in individuals possessing 1 or 2 ε4 alleles. The relationship between GFAP and Aβ was not strong compared with the variation in GFAP levels, but there was a small non-significant ($p = 0.12$) increase in GFAP levels by *APOE* status after adjusting for Aβ (fig. 4). *APOE* did not affect levels of GFAP after adjusting for Braak stage ($p = 0.4$) or AT8 immunostaining ($p = 0.36$).

Discussion

GFAP is a well-characterised marker for assessment of astrocyte reactivity, its expression induced by a variety of processes relevant to neurodegeneration [28, 29]. It increases with age [30] and some studies have shown an increase in association with dementia [16]. We have recently shown variation in the pattern of GFAP expression in the temporal cortex in a population-based sample and we now report wide variation in population expression of GFAP using a quantitative ELISA-based method. Other studies of astrocytes in ageing brain have used S100B as

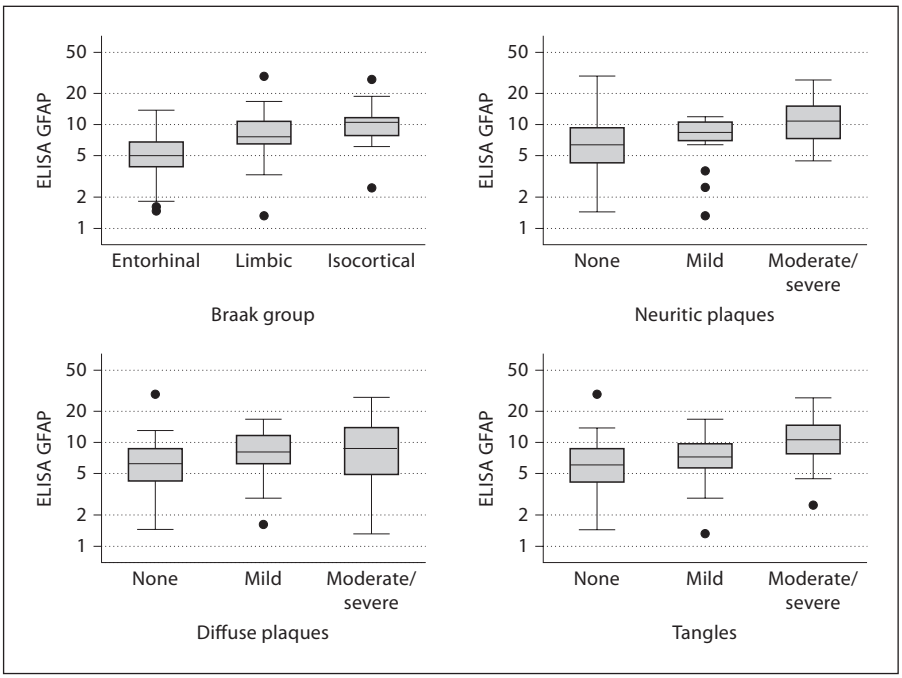

Fig. 2. Box-whisker plots of ELISA-measured GFAP according to Braak group and local measures of Alzheimer-type pathology.

Table 1. ELISA GFAP levels according to Braak stage and dementia status

	Entorhinal			Limbic			Isocortical			Total[1]		
	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD
No dementia	17	6.3	2.8	9	8.0	2.1	1	10.7	–	27	7.0	2.7
Dementia	7	5.6	5.0	26	9.3	5.6	13	11.5	6.3	47	9.3	5.9
Total[2]	25	5.9	3.5	36	8.9	4.9	14	11.5	6.0	76	8.4	5.1
	n	med	IQR	n	med	IQR	n	med	IQR	n	med	IQR
No dementia	17	5.7	2.4	9	7.9	2.3	1	10.7	–	27	6.7	4.4
Dementia	7	3.5	10.2	26	7.5	5.7	13	10.4	3.9	47	7.8	6.1
Total[2]	25	5.0	2.8	36	7.7	4.3	14	10.5	3.9	76	7.3	5.8
F-test for difference in variance	F(6, 16) = 3.79 p = 0.031			F(25, 8) = 4.85 p = 0.026						F(46, 26) = 2.70 p = 0.008		

The mean, standard deviation (SD), median (med) and interquartile range (IQR) of GFAP levels by dementia status and Braak tangle stage. Braak stages area combined into the following groups: entorhinal (Braak 0–II), limbic (III–IV) and neocortical (V–VI) stages. Tests for difference in variance were calculated using log-transformed values of GFAP.

[1] Includes 1 case with unknown Braak stage.

[2] Includes 2 cases with unknown dementia at death.

a marker, a neurotrophic cytokine that shows age-related increases and which may be important in the pathogenesis of AD [9, 31]. It is not astrocyte-specific, however, and may behave differently to GFAP. In a mouse senescence model, GFAP but not S100B increased with ageing [32]. These markers therefore provide different, complementary insights into changes in astroglial phenotype. In our recent study, immunohistochemistry for S100 on the temporal cortex did not show the same degree of population variation as GFAP, although the quantitative limits

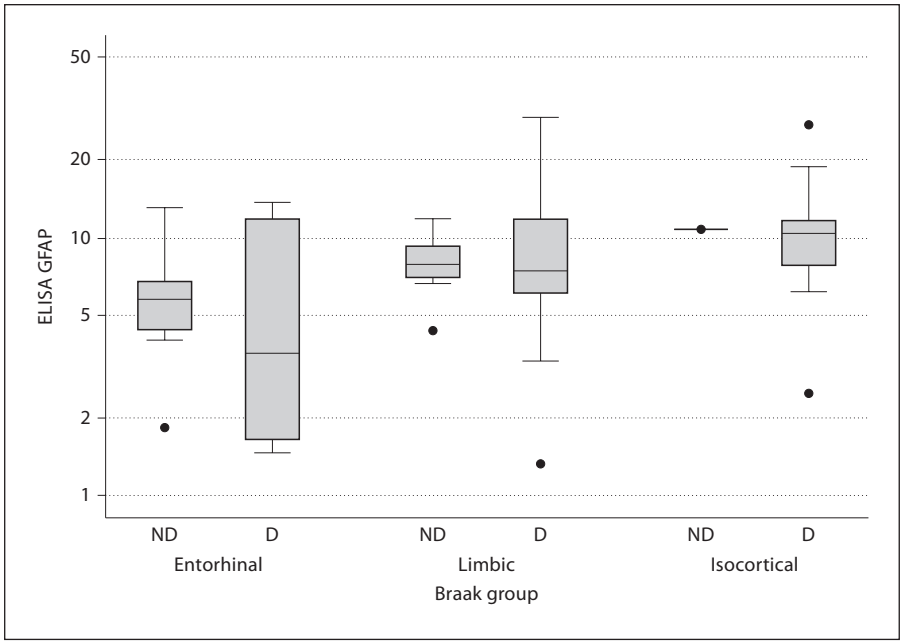

Fig. 3. Box plot showing the relationship between dementia and ELISA GFAP (on a log scale) stratified by Braak stage. ND = Non-demented; D = demented.

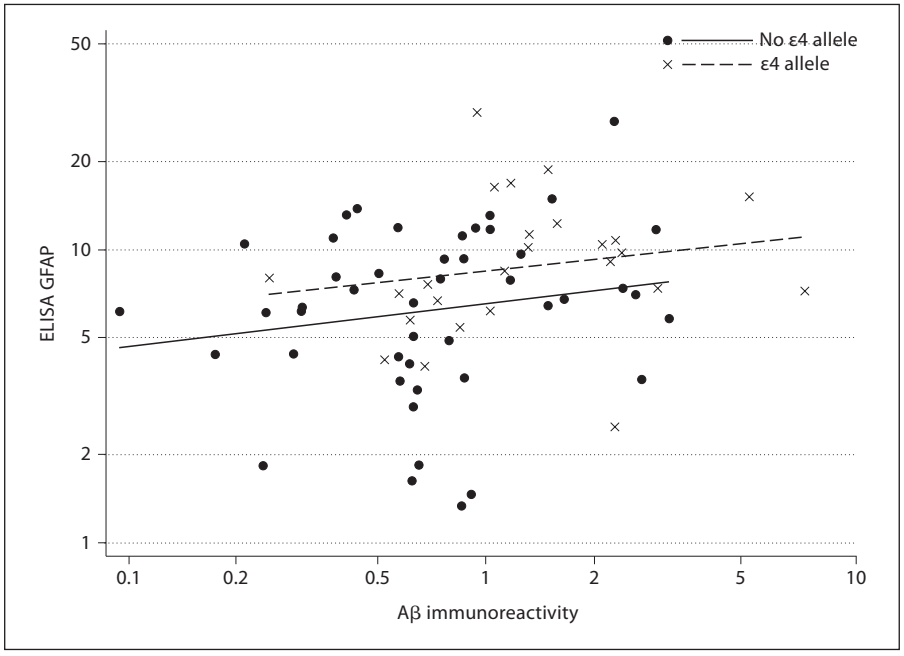

Fig. 4. Scatter plot showing the effect of Aβ levels on GFAP stratified by possession of the *APOE* ε4 allele.

of our approach should be noted [17]. We therefore selected GFAP for this study in the lateral temporal cortex. This is a cortical area commonly involved in Alzheimer-type pathology and previous work has suggested that the glial response is most prominent in the temporal region [21]. The measures reported in this study are of total GFAP, but splice variants of GFAP are described, which may show differences in localisation and function [17, 33–35]. GFAP may also undergo caspase cleavage. This appears to be localised particularly to regions rich in Alzheimer-type pathology and to be associated with astrocyte damage [36]. The effects of variations in isoform expression on astrocyte phenotype and function in brain ageing are currently known.

The causes of the wide variation in the astroglial response in brain ageing, its functional consequences and

its interactions with AD and other ageing pathologies are key questions. In our study, and others, astroglial reactivity was associated with Alzheimer-type pathology, which may be an important, but not exclusive, driver of the glial reaction. GFAP expression increased with Braak stage and local (temporal cortex) measures of Alzheimer-type pathology. These relationships were attenuated, but remained significant after adjusting for age, which does not account for this relationship. It should be noted that immunohistochemical assessments were performed on lateral temporal cortex contralateral to the frozen samples used for the ELISA. The development of plaques and tangles, at least in AD, tends to be symmetrical in the temporal cortex [37], but this is a limitation of this study. The possibility of anatomical variation of GFAP levels within an individual, both within and across hemispheres, has not been formally addressed either; in future studies, it may be valuable to take measurements from more than one area. In addition, immunohistochemistry is not as quantitative as an ELISA [20].

Astrocytes respond to Alzheimer-type lesions, which are one driver of astrogliosis. They are a target of Aβ toxicity; this may have secondary effects on neurons through loss of support, free radical and cytokine production [7, 8]. Activated glia also sensitise neurons to injurious stimuli [38]. Astrocytes are recruited to plaques, involved in plaque clearance and may be injured in this process [5, 39]. Increased GFAP expression [17] shows a stronger relation to compact than to diffuse plaques. Reactive astrocytes also appear to associate with compact plaques, but not with diffuse amyloid deposits. This may represent a response to the more fibrillar forms of Aβ, to plaque-associated inflammatory mechanisms or to processes associated with neuritic damage.

In this study, we show population variation in the astrocytic response in those with significant burdens of Alzheimer-type pathology, which may mediate a varying effect on the brain, but also considerable variation in GFAP in individuals with little Alzheimer-type pathology, even those who are not demented. It has been suggested that astrogliosis is a late response, compared to the microglial response [4]. In this population-based sample, however, elevation of GFAP was seen in association with earlier stages of Alzheimer-type pathology, rising in the limbic stages (at a stage before tangle appearance) and increasing further in the isocortical stages. Astrogliosis may therefore commence as an early event in AD, and also occurs early in individuals with Down's syndrome, in whom development of Alzheimer-type pathology is observed [9]. This raises the question of the causes of astroglial pathology in ageing, suggesting that established AD lesions, recognisable by classical neuropathology methods, are not the only drivers, especially in early disease or 'normal ageing'. Astrocytes may respond to preclinical AD molecular pathology, and the potential role of oligomeric aggregates is of interest. In model systems, subtle nerve terminal damage can trigger a gliotic response [40]. This may suggest that in some individuals an astrocyte response may occur to more subtle neuronal or synaptic pathology. Other candidate drivers might include age-related oxidative stress and DNA damage, which can occur 'before' the development of significant Alzheimer-type pathology [41]. Microglial activity is also upregulated in brain ageing [9, 42] and can activate astrocytes. Additionally, senescence, which can be related to DNA damage [43], is an unexplored factor in glial ageing.

This suggests a potentially complex interaction model for astrocyte involvement in brain ageing, whereby (1) the astrocyte cellular pathology or response, due to ageing brain processes or early AD molecular pathology, leads to altered function, (2) altered astrocyte function interacts with developing Alzheimer molecular pathology to affect lesion progression, (3) altered astrocyte function affects the outcome for Alzheimer-type pathology on cognitive function and (4) astrocyte function is in turn altered by Alzheimer-type pathology. Each of these phases may be affected by variation in how astrocytes respond within the population. The wide variation in GFAP expression in both demented and non-demented individuals, and at all stages of disease, supports a role for changes in astrocyte phenotype as a contributory factor to the population variation in ageing brain outcomes.

This suggests that astroglial reactions may be an early marker of neurodegenerative processes and a potential predictor of progression. Variation in astrocyte response could also be a factor in determining the likelihood of becoming demented for given loads of Alzheimer-type pathology. We did not, however, demonstrate that GFAP expression was higher in demented compared to non-demented individuals at given Braak stages. This is in contrast to findings in the Honolulu-Asia Aging Study [16]. That study examined 4 cortical brain areas in a larger number of cases (n = 204) and the relationship was found in 3 areas (including temporal), whereas we have examined a single area. However, the reason for the difference in findings between these two studies in different populations is currently unclear. Other factors need to be considered in further addressing this hypothesis. The ELISA method does not take into account variations in relative

contributions of different cortical layers to the upregulation (e.g. subpial vs. other cortical layers) nor in different iso- or cleaved forms of GFAP. GFAP also reflects only the hypertrophy aspect of astrocytic pathology. The regulation and functional roles of gliosis have yet to be defined, although transgenic approaches are beginning to address these questions [44–46]. Other markers may better reflect other aspects of an altered astrocyte phenotype, e.g. DNA damage, loss of function. We have recently shown variation in loss of the glutamate transporter EAAT2 [17], and there is evidence of diversity in astrocytic phenotype [47], the influence of which in the ageing response has yet to be addressed.

Of note, we showed significantly wider variance in GFAP levels in demented compared to non-demented individuals overall and at entorhinal and limbic stages. This was not solely due to higher extremes of GFAP in some cases due to Alzheimer-type pathology, but was also seen in demented cases with lower levels of GFAP compared to the non-demented. Within the entorhinal stage there were limited numbers (n = 7) in the demented group, and there was only one case without dementia at the isocortical stage. Even so, this result reached significance and was a consistent pattern. The basis of this finding is unclear at present.

Astrocytes can clear deposited Aβ [10] in an apoE-dependent manner [48], so *APOE* genotype may contribute to the variation in astrocyte response. We have found a non-significant trend to greater GFAP expression in relation to Aβ in those bearing an ε4 allele, but the difference was small compared to the variation in GFAP expression. Assessment of Aβ in our study was immunohistochemical and contralateral to the tissue used for ELISA measurement of GFAP. This question would be worth further investigation using more sensitive and quantitative measures of Aβ species.

In conclusion, the variation in GFAP expression indicates a wide population variation in astrogliosis in ageing. This elevation precedes frank AD and is not entirely accounted for by Alzheimer-type pathology. Astrocyte responses and cellular pathology are important questions in brain ageing as, not only may changes in astrocytes modulate the development and effects of Alzheimer-type pathology, they may also affect response to novel therapies. Inflammatory mechanisms are suggested to be important in AD development and there is interest in their therapeutic modulation [49]. Non-steroidal anti-inflammatory agents, which some studies suggest may have a therapeutic effect, appear to lower astrocyte counts in AD [50]. Interindividual variation in astroglial responses are worth characterising as determinants of cognitive outcomes in ageing and of responses to therapeutic interventions in dementia.

Acknowledgements

This study was supported by grants from the Alzheimer's Research Trust (PG2006/6) and the UK Medical Research Council. We are grateful to the respondents to the CFAS study, their families and their carers for agreement to participate in the brain donation programme.

References

1 Hardy J, Selkoe D: The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002;297:353–356.

2 Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC-CFAS): Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. Lancet 2001;357:169–175.

3 Maragakis N, Rothstein J: Mechanisms of disease: astrocytes in neurodegenerative disease. Nat Clin Pract Neurol 2006;2:679–689.

4 Vehmas A, Kawas C, Stewart W, Troncoso J: Immune reactive cells in senile plaques and cognitive decline in Alzheimer's disease. Neurobiol Aging 2003;24:321–331.

5 Nagele R, Wegiel J, Venkataraman V, Iamki H, Wang KC, Wegiel J: Contribution of glial cells to the development of amyloid plaques in Alzheimer's disease. Neurobiol Aging 2004;25:663–674.

6 Oide T, Kinoshita T, Arima K: Regression of senile plaques in the natural course of Alzheimer's disease. Neuropathol Appl Neurobiol 2006;32:539–556.

7 Meda L, Baron P, Scarlato G: Glial activation in Alzheimer's disease: the role of Aβ and its associated proteins. Neurobiol Aging 2001; 22:885–893.

8 Abramov A, Canevari L, Duchen M: Changes in intracellular calcium and glutathione in astrocytes as the primary mechanism of amyloid toxicity. J Neurosci 2003;23:5088–5095.

9 Mrak R, Griffin W: Glia and their cytokines in progression of neurodegeneration. Neurobiol Aging 2005;26:349–354.

10 Wyss-Coray T, Loike J, Brionne T, Lu E, Anankov R, Yan F, Silverstein S, Husemann J: Adult mouse astrocytes degrade amyloid-Aβ in vitro and in situ. Nat Med 2003;9:453–457.

11 Volterra A, Meldolesi J: Astrocytes, from brain glue to communication elements: the revolution continues. Nat Rev Neurosci 2005;6:626–640.

12 Pekny M, Nilsson M: Astrocyte activation and reactive gliosis. Glia 2005;50:427–434.

13 Jing R, Wilhelmsson U, Goodwill W, Li L, Pan Y, Pekny M, Skalli O: Synemin is expressed in reactive astrocytes in neurotrauma and interacts differentially with vimentin and GFAP intermediate filament networks. J Cell Sci 2007;120:1267–1277.

14 Morgan T, Rozovsky I, Goldsmith S, Stone D, Yoshida T, Finch C: Increased transcription of the astrocyte gene GFAP during middle-age is attenuated by food restriction: implications for the role of oxidative stress. Free Radic Biol Med 1997;23:524–528.
15 Ingelsson M, Fukomoto H, Newell K, Growdon J, Hedley-Whyte E, Frosch M, Albert M, Hyman B, Irizarry M: Early Aβ accumulation and progressive synaptic loss, gliosis, and tangle formation in AD brain. Neurology 2004;62:925–931.
16 Kashon M, Ross G, O'Callaghan J, Miller D, Petrovitch H, Burchfield C, Sharp D, Markesbery W, Davies D, Hardman J, Nelson J, White L: Associations of cortical astrogliosis with cognitive performance and dementia status. J Alzheimers Dis 2004;6:595–604.
17 Simpson J, Ince P, Lace G, Forster G, Shaw P, Matthews F, Savva G, Brayne C, Wharton S; on behalf of the MRC Cognitive Function and Ageing Neuropathology Study Group: Astrocyte phenotype in relation to Alzheimer-type pathology in the ageing brain. Neurobiol Aging 2008, E-pub ahead of print.
18 Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC-CFAS): Cognitive function and dementia in six areas of England and Wales: the distribution of MMSE and prevalence of GMS organicity level in the MRC CFA study. Psychol Med 1998;28:319–335.
19 Braak H, Braak E: Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 1991;82:239–259.
20 McLendon R, Bigner D: Immunohistochemistry of the glial fibrillary acidic protein: basic and applied considerations. Brain Pathol 1994;2:221–228.
21 Ross G, O'Callaghan J, Sharp D, Petrovich H, Miller D, Abbott R, Nelson J, Launer L, Foley D, Burchfield C, Hardman J, White L: Quantification of regional glial fibrillary acidic protein levels in Alzheimer's disease. Acta Neurol Scand 2003;107:318–323.
22 Mirra S, Heyman A, McKeel D, Sumi S, Crain B, Brownlee L, Vogel F, Hughes J, Van Belle G, Berg L: The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). 2. Standardisation of the neuropathologic assessment of Alzheimer's disease. Neurology 1991;41:479–486.
23 Braak H, Alafuzoff I, Arzberger T, Kretaschmar H, del Tredici K: Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol 2006;112:389–404.
24 O'Callaghan J: Quantification of glial fibrillary acidic protein: comparison of slot-immunobinding assays with a novel sandwich ELISA. Neurotoxicol Teratol 1991;13:275–281.
25 O'Callaghan J: Measurement of glial fibrillary acidic protein; in Costa L (ed): Current Protocols in Toxicology. New York, Wiley, 2002, pp 12.81.11–12.81.12.
26 Nicoll J, Burnett C, Love S, Graham D, Dewar D, Ironside J, Stewart J, Vinters H: High frequency of apolipoprotein E epsilon 2 allele in hemorrhage due to cerebral amyloid angiopathy. Ann Neurol 1997;41:716–721.
27 Wenham P, Price W, Blandell G: Apolipoprotein E genotyping by one-stage PCR. Lancet 1991;337:1158–1159.
28 Laping N, Teter B, Nichols N, Rozovsky I, Finch C: Glial fibrillary acidic protein: regulation by hormones, cytokines, and growth factors. Brain Pathol 1994;4:259–275.
29 Vinters H, Kleinschmidt-Demasters B: General pathology of the central nervous system; in Love S, Louis D, Ellison D (eds): Greenfield's Neuropathology. London, Hodder Arnold, 2008, pp 8–22.
30 Nichols N, Day J, Laping N, Johnson S, Finch C: GFAP mRNA increases with age in rat and human brain. Neurobiol Aging 1993;14:421–429.
31 Sheng J, Mrak R, Rovnaghi C, Kozlowska E, Van Eldik L, Griffin W: Human brain S100β and S100β mRNA expression increases with age: pathogenic implications for Alzheimer's disease. Neurobiol Aging 1996;17:359–363.
32 Wu Y, Zhang AQ, Yew DT: Age related changes of various markers of astrocytes in senescence-accelerated mice hippocampus. Neurochem Int 2005;46:565–574.
33 Roelofs R, Fischer D, Houtman S, Sluijs J, Van Haren W, Van Leeuwen F, Hol E: Adult human subventricular, subgranular, and subpial zones contain astrocytes with a specialized intermediate filament cytoskeleton. Glia 2005;52:289–300.
34 Nielsen A, Holm I, Johansen M, Bonven B, Jorgensen P, Jorgensen A: A new splice variant of glial fibrillary acidic protein, GFAPε, interacts with the presenilin proteins. J Biol Chem 2002;277:29983–29991.
35 Blechinberg J, Holm I, Nielsen K, Jensen T, Jorgensen A, Nielsen A: Identification and characterization of GFAPκ, a novel glial fibrillary acidic protein isoform. Glia 2007;55:497–507.
36 Mouser P, Head E, Ha KH, Rohn T: Caspase-mediated cleavage of glial fibrillary acidic protein within degenerating astrocytes of the Alzheimer's disease brain. Am J Pathol 2006;168:936–946.
37 Moossy J, Zubenko G, Martinez A, Rao G: Bilateral symmetry of morphologic lesions in Alzheimer's disease. Arch Neurol 1988;45:251–254.
38 Mander P, Borutaite V, Moncada S, Brown G: Nitiric oxide from inflammatory-activated glia synergizes with hypoxia to induce neuronal death. J Neurosci Res 2004;79:208–215.
39 Kobayashi K, Hayashi M, Nakano H, Shimazaki M, Sugimori K, Koshino Y: Correlation between astrocyte apoptosis and Alzheimer changes in gray matter lesions in Alzheimer's disease. J Alzheimers Dis 2004;6:623–632.
40 O'Callaghan J, Sriram K: Glial fibrillary acidic protein and related glial proteins as biomarkers of neurotoxicity. Expert Opin Drug Saf 2005;4:433–442.
41 Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj E, Jones P, Ghanbari H, Wataya T, Shimohama S, Chiba S, Atwood C, Petersen R, Smith M: Oxidative damage is the earliest event in Alzheimer disease. J Neuropathol Exp Neurol 2001;60:759–767.
42 Sheng J, Mrak R, Griffin W: Enlarged and phagocytic, but not primed, interleukin-1α-immunoreactive microglia increase with age in normal brain. Acta Neuropathol 1998;95:229–234.
43 Von Zglinicki T, Saretzki G, Ladhoff J, d'Adda di Fagagna F, Jackson S: Human cell senescence as a DNA damage response. Mech Ageing Dev 2005;126:111–117.
44 Herrmann J, Imura T, Song B, Qi J, Ao Y, Nguyen T, Korsak R, Takeda K, Akira S, Sofroniew M: STAT3 is a critical regulator of astrogliosis and scar formation after spinal cord injury. J Neurosci 2008;28:7231–7243.
45 Correa-Cerro L, Mandell J: Molecular mechanisms of astrogliosis: new approaches with mouse genetics. J Neuropathol Exp Neurol 2007;66:169–176.
46 Lepore A, Dejea C, Carmen J, Rauck B, Kerr D, Sofroniew M, Maragakis N: Selective ablation of proliferating astrocytes does not affect disease outcome in either acute or chronic models of motor neuron degeneration. Exp Neurol 2008;211:423–432.
47 Lovatt D, Sonnewald U, Waagepetersen H, Schousboe A, He W, Lin JC, Han X, Takano T, Wang S, Sim F, Goldman S, Nedergaard M: The transcriptome and metabolic gene signature of protoplasmic astrocytes in the adult murine cortex. J Neurosci 2007;27:12255–12266.
48 Koistinaho M, Lin S, Wu X, Esterman M, Koger D, Hanson J, Higgs R, Liu F, Malkani S, Bales K, Paul S: Apolipoprotein E promotes astrocyte colocalisation and degradation of deposited amyloid-β peptides. Nat Med 2004;10:719–726.
49 Wyss-Coray T: Inflammation in Alzheimer disease: driving force, bystander or beneficial response? Nat Med 2006;12:1005–1015.
50 Alafuzoff I, Overmyer M, Helisalmi S, Soininen H: Lower counts of astroglia and activated microglia in patients with Alzheimer's disease with regular use of non-steroidal anti-inflammatory drugs. J Alzheimers Dis 2000;2:37–46.