Am J Neurodegener Dis 2014;3(1):19-32
www.AJND.us /ISSN:2165-591X/AJND1402006

Original Article

Development, appraisal, validation and implementation of a consensus protocol for the assessment of cerebral amyloid angiopathy in post-mortem brain tissue

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Received January 14, 2014; Accepted February 18, 2014; Epub April 2, 2014; Published April 12, 2014

Abstract: In a collaboration involving 11 groups with research interests in cerebral amyloid angiopathy (CAA), we used a two-stage process to develop and in turn validate a new consensus protocol and scoring scheme for the assessment of CAA and associated vasculopathic abnormalities in post-mortem brain tissue. Stage one used an iterative Delphi-style survey to develop the consensus protocol. The resultant scoring scheme was tested on a series of digital images and paraffin sections that were circulated blind to a number of scorers. The scoring scheme and choice of staining methods were refined by open-forum discussion. The agreed protocol scored parenchymal and meningeal CAA on a 0-3 scale, capillary CAA as present/absent and vasculopathy on 0-2 scale, in the 4 cortical lobes that were scored separately. A further assessment involving three centres was then undertaken. Neuropathologists in three centres (Bristol, Oxford and Sheffield) independently scored sections from 75 cases (25 from each centre) and high inter-rater reliability was demonstrated. Stage two used the results of the three-centre assessment to validate the protocol by investigating previously described associations between *APOE* genotype (previously determined), and both CAA and vasculopathy. Association of capillary CAA with or without arteriolar CAA with *APOE* ε4 was confirmed. However *APOE* ε2 was also found to be a strong risk factor for the development of CAA, not only in AD but also in elderly non-demented controls. Further validation of this protocol and scoring scheme is encouraged, to aid its wider adoption to facilitate collaborative and replication studies of CAA.

Keywords: Angiopathy, amyloid, dementia, Delphi, validation, *APOE*, CAA, consensus, parenchymal, meningeal

Introduction

Cerebral amyloid angiopathy (CAA) is a disease in which amyloid is deposited within the walls of meningeal and parenchymal blood vessels – predominantly arteriolar and scanty venous deposits but occasionally involving capillaries. Most cases are sporadic and result from the accumulation of the Alzheimer's disease (AD)-associated amyloid-β peptide (Aβ), although rare autosomal dominant mutations of a number of genes produce vascular accumulation of amyloid composed of other proteins such as cystatin C, ABri and ADan. Sporadic CAA affects approximately 30% of the neurologically-normal elderly [1] and 90-100% of those with AD [2]. It is most often asymptomatic but can cause or contribute to dementia [3], cerebral haemorrhage [4, 5] and ischaemic damage [2, 6-8].

The aetiology of sporadic CAA is unclear. However extensive work carried out by Weller [9] and others over the last decade suggests that sporadic CAA is a protein-elimination-fail-

ure arteriopathy [10-12], whereby impairment of perivascular 'lymphatic' drainage, endothelial transport and degradation of Aβ by cerebrovascular smooth muscle cells and perhaps perivascular macrophages participate (to varying degrees) in the perivascular build up of Aβ and development of CAA. Contributing factors are likely to include the effects of age on the thickness, composition and compliance of the perivascular basement membranes of cerebral arterioles along which interstitial fluid drains from the brain. Weller *et al.* proposed that this drainage is driven by the systolic pulsation of the blood vessels [13]. Genetic factors are probably also important, particularly the *APOE* genotype, which was reported to influence the development of CAA by altering the relative proportions of Aβ40:Aβ42 [14] and is also associated with the level and activity of the Aβ-degrading enzyme neprilysin in cerebrovascular smooth muscle cells [11, 15]. Capillary CAA on the other hand has been suggested to be primarily caused by reduced transendothelial clearance of Aβ-APOE4 protein complexes [16, 17].

Several early studies found a relationship between *APOE* genotype and the severity of CAA in patients with AD, patients with one or two copies of the *APOE* ε4 allele having more severe CAA than those without an ε4 allele [4, 18, 19], although others found that there was no relationship [20, 21]. A recent systematic review and meta-analysis of all published studies on *APOE* and CAA supported the association for the presence of a dose dependent association between *APOE* ε4 and sporadic CAA [22, 23]. Thal *et al.* [16, 17] has also reported that sporadic CAA could be subdivided into two distinct entities: (A) type 1 CAA, characterised by the presence of capillary Aβ with or without Aβ deposition in arterial vessels; (B) type 2 CAA in which Aβ deposition is restricted to arterial vessels.

These authors found that regardless of the presence or absence of AD, only CAA type 1 was strongly associated with *APOE* ε4 [16, 17], whereas type 2 was associated with *APOE* ε2. *APOE* genotype was also reported to be associated with vasculopathic abnormalities and cerebral haemorrhage in CAA. Nicoll *et al.* found that *APOE* ε2 allele was significantly over-represented in CAA-associated cerebral haemorrhage [5]. Greenberg *et al.* concluded that *APOE* ε4 increased CAA severity by enhancing Aβ deposition within cerebral blood vessels whilst *APOE* ε2 promoted the vasculopathic changes which led to vessel rupture [24].

Rationale

Sporadic CAA is a major focus of research by many groups in several countries. CAA has considerable co-morbidity with AD, the most common form of dementia and one of the largest health care challenges of modern times. It is therefore somewhat surprising that there is no generally agreed protocol for the assessment of CAA in post-mortem brain tissue [25-27]. In contrast there are widely used protocols for the diagnosis and staging of AD pathology [28] and these have greatly facilitated AD research. The need to develop a broadly-accepted protocol for the assessment of CAA, to facilitate research collaboration and improve reproducibility of findings between different groups, was strongly endorsed at the 2nd International Conference on CAA in Newcastle, UK in 2002. The present paper describes the use of the Delphi method co-ordinated by the Dementia Research Group in the University of Bristol to develop, appraise, validate and apply such a protocol in collaboration with other research groups in the United Kingdom, mainland Europe, North America and Japan. The process is outlined in **Figure 1**.

Methods

Stage 1 – development of protocol

A literature review was first carried out to identify research groups that had published on CAA in post-mortem human brain tissue. Sixteen research groups were contacted by email and asked if they were they willing to participate in the development of a consensus protocol for assessment of CAA. Representatives from 12 of the 16 groups consented. A Delphi-style survey [29, 30] was initiated using an online survey tool (Bristol Online Surveys (http://www.survey.bris.ac.uk/)) hosted by the University of Bristol. A uniform resource locator (URL) weblink specific to each round of the survey was provided by email to the participants and all questions were completed online completely anonymised. The protocol development phase took 6 rounds to complete.

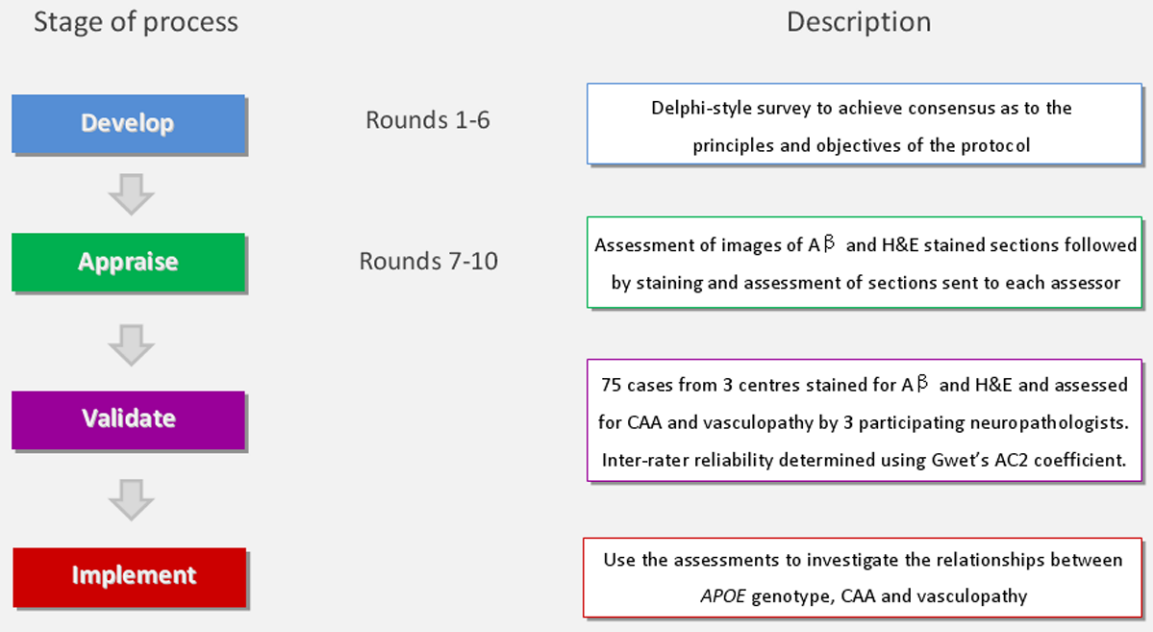

Figure 1. Flow diagram of the CAA consensus protocol process. A protocol was developed by CAA researchers using a modified Delphi method before being implemented by participants using images and sections of Aβ immunolabelled and H&E stained brain sections. The protocol was then validated by the assessment of 75 cases by 3 neuropathologists and inter-rater reliability determined. The results from the validation process were then used to investigate the relationship between *APOE* genotype, CAA severity and vasculopathy.

Table 1. Principles suggested and scored by participants

Principle	Cumulative score
Enable valid comparisons between cohorts	33
Subject to scientific validation	33
Achieve high intra- and inter-rater reliability	32
User-friendly and easy to apply	30
Include several relevant regions of brain	29
Should not require specialised equipment	29
Record association with other pathological findings	25
Avoid prior assumptions about biological relevance of CAA (i.e. avoid assessing severity according to the absence of presence of findings such as infarcts or haemorrhage)	25
Assessment of CAA based on labelling with antibodies	24
Highly sensitive to detection of mild CAA	22
Relate score to clinical as well as pathological features	19
Based on same blocks as used for CERAD assessment	18
Demonstrate amyloid nature of vascular deposits, in addition to Aβ on immunohistochemistry	15

In round 1, participants were asked to list the principles and objectives that they believed should form the structure of the protocol. In round 2, participants were asked to score each principle or objective and for its perceived importance (**Tables 1** and **2**). In round 3 the cumulative scores were circulated to all participants, who were asked again, in the light of these scores, for their views, particularly in relation to potentially contradictory principles or objectives (e.g. that the assessment protocol should (i) avoid prior assumptions about the relationship of CAA to infarcts or haemorrhage, but (ii) integrate clinical and radiological information in the assessment). This yielded agreement which implied that the highest scores should determine the principles and objectives to be adopted for the assessment protocol. In

Table 2. Objectives suggested and scored by participants

Objective	Cumulative score
Separate assessment of leptomeningeal and parenchymal CAA	28
Clear indication in assessment summary of regions affected	28
Record 'secondary' pathologies such as fibrinoid necrosis	26
Obtain an overall score of CAA severity	26
Categorise according to type of affected vessel in each region (i.e. arterioles and capillaries)	26
Allow for grading of CAA in biopsy as well as autopsy material	22
Allow for integration of clinical and radiological information in the assessment	18
Score severity according to maximum severity observed in any region	17
Take *APOE* genotype into account	13

Table 3. Hybrid protocol for scoring CAA and CAA-associated vasculopathy

Score	Parenchymal CAA	Meningeal CAA	Capillary CAA	Vasculopathy
0	Absent	Absent	Absent	Absent
1	Scant Aβ deposition	Scant Aβ deposition	Present	Occasional vessel
2	Some circumferential Aβ	Some circumferential Aβ		Many vessels
3	Widespread circumferential Aβ	Widespread circumferential Aβ		

Table 4. Mean age, SD and minimum and maximum ages of AD and control cases in the three participating cohorts

Cohort	N	Mean	SD	Min	Max
Oxford - AD	20	80.9	6.8	66	91
Oxford - control	5	83.2	7.3	77	92
Oxford - total	25	81.4	6.8	66	92
CFAS - AD	11	89.5	6.4	79	102
CFAS - control	4	83.0	6.3	76	91
CFAS - total	15	87.7	6.8	76	102
Bristol - AD	20	78.2	10.2	60	95
Bristol - control	5	87.6	5.6	80	94
Bristol - total	25	80.0	10.1	60	95
Total - AD	51	81.7	9.1	60	102
Total - control	14	84.7	6.32	76	94
Total	65	82.3	8.7	60	102

round 4 participants were asked to consider more detailed aspects of an assessment protocol: to indicate what antibodies or stains they would use, at what magnification they would make the assessments, and to submit a sample protocol taking account of the principles and objectives previously agreed. In round 5 all sample protocols were fed back to the participants, who were asked to score each one according to how well it was thought to fulfil the agreed principles and objectives. The protocols were then ranked according to cumulative score, the common elements were extracted from the protocols with the highest scores and a hybrid protocol was formulated (**Table 3**). In round 6 the hybrid protocol was sent to all participants, who were asked to comment on its suitability. All participants agreed that the suggested protocol was suitable for the assessment of CAA.

Stage 2 – appraisal

To assess the ease of use of the protocol and the reproducibility of the assessments, sections from 3 cases of CAA were (i) stained with Harris's haematoxylin and eosin (H&E); (ii) labelled with an agreed antibody to Aβ (Signet 4G8, distributed by Covance, UK; 1:16,000 overnight) by standard avidin-biotin-peroxidase immunohistochemistry after pre-treatment with 100% formic acid for 20 minutes, and (iii) sections were also stained with Martius scarlet blue (MSB) and phosphotungstic acid/haematoxylin (PTAH) when fibrinoid necrosis was suspected. Many photomicroscope images of the stained sections were circulated to the participants for scoring of the severity of CAA and for comment on the presence or absence or vasculopathy and capillary CAA, according to the agreed protocol. Scores were collated and fed back to the participants. The results showed that inter-rater reliability was good when scoring meningeal, parenchymal and capillary CAA

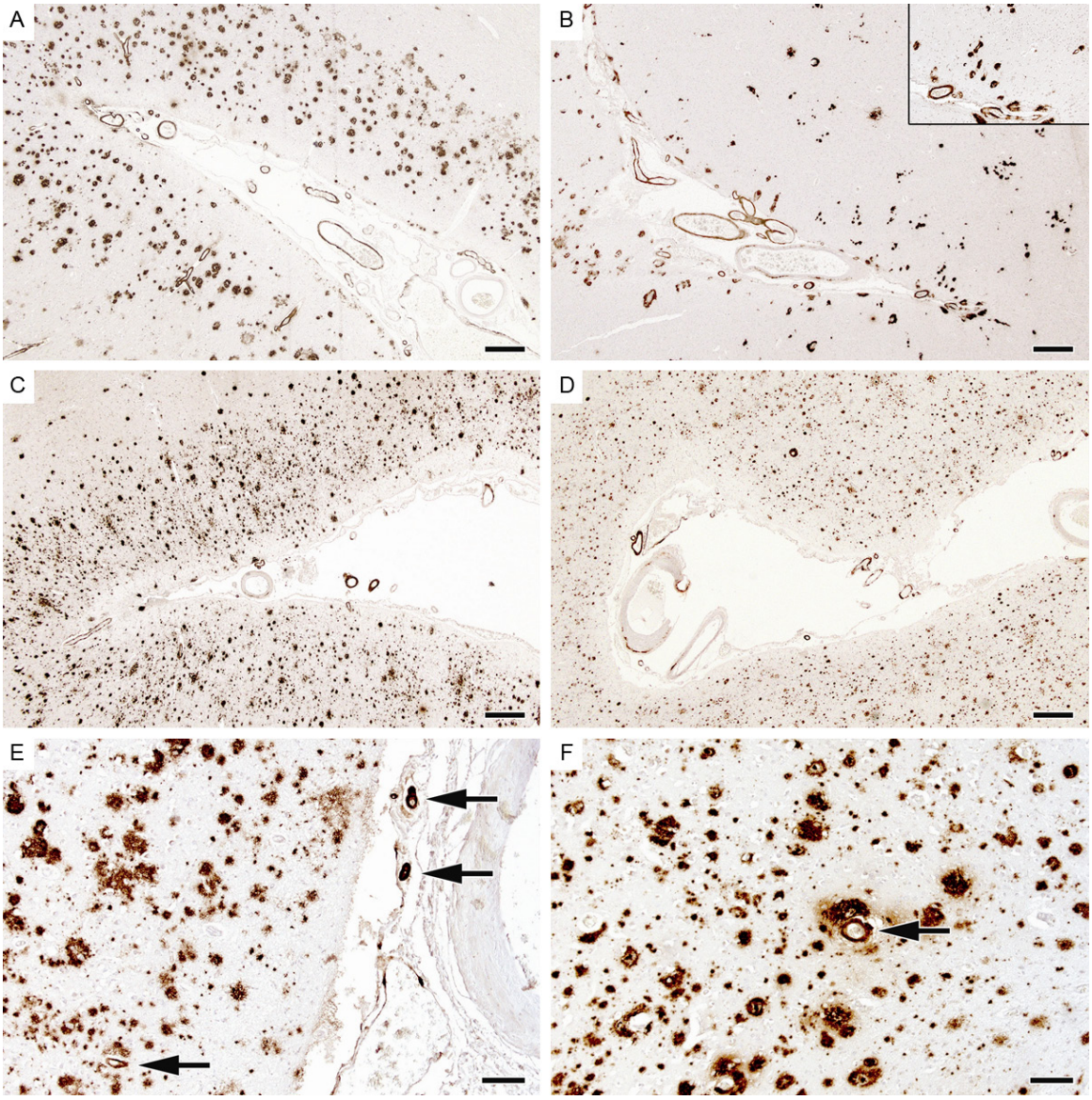

Figure 2. CAA severity scores – representative examples. The sections have been immunolabelled for Aβ. A and B. Illustrate an arteriolar CAA severity score of 3 in the leptomeninges and cerebral cortex, with Aβ in most arterioles and circumferential deposition in many. C and D. Illustrate a CAA severity score of 2 in both meninges and parenchyma. Several arterioles show Aβ deposition, in some cases circumferential. E and F. Illustrate sections in which the arteriolar CAA score was 1. Most fields lacked any vascular Aβ; the arrows indicate the only Aβ-positive vessels within the sections. The scale bars in A-D represent 400 μm, those in E and F 100 μm.

but there was variability in the scoring of vasculopathy. Participants were asked to comment on how the identification of vasculopathy could be improved and the responses were circulated.

Unstained formalin-fixed paraffin-embedded sections of frontal and temporal cortex from 3 AD cases with differing CAA severity were then sent to all participants for staining (Aβ and H&E) and scoring. Once again the consistency of assessment of parenchymal and meningeal CAA was good. However there was variation in the detection of capillary CAA and vasculopathic abnormalities.

The 3rd International Conference on CAA in Reykjavik, Iceland in 2007 provided an opportunity for the study to be discussed in an open meeting chaired by KC, who presented the results of the appraisal rounds. Most of the researchers who were involved in the study, as

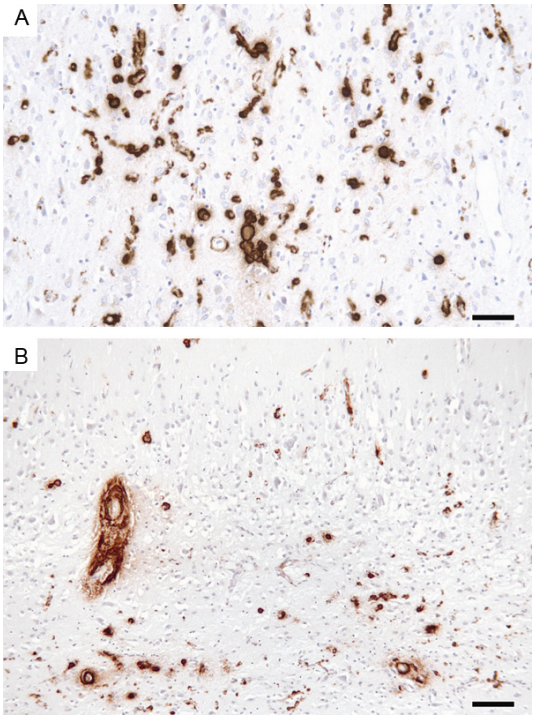

Figure 3. Aβ immunolabelling of capillary CAA in the occipital cortex. A. Florid capillary CAA. B. Capillary CAA with concomitant severe arteriolar CAA. Sections with any capillary CAA score 1, those with no capillary CAA score zero. The scale bars represent 50 μm in A, 100 μm in B.

well as some who were not, discussed ways to improve the recognition of capillary CAA and to score and record vasculopathic abnormalities.

In the final round of the protocol appraisal, opinions were solicited on the stains to be used to detect capillary CAA and fibrinoid necrosis. Agreement was reached that the pan-Aβ antibody was adequate to detect capillary CAA. Participants felt that fibrinoid necrosis was too infrequent to warrant the routine use of MSB or PTAH to detect it but that either of these stains should be used when fibrinoid necrosis was suspected on examination of H&E-stained sections.

Stage 3 – validation

Following the development of the protocol, a Network Cooperation grant was obtained from the Alzheimer's Research Trust (now Alzheimer's Research UK) to carry out a validation study of the consensus protocol. Three centres with access to post-mortem brain tissue (Bristol, Oxford and Sheffield (CFAS cohort)) agreed to take part in the validation study. The intention was to examine 20 AD (CERAD definite, Braak tangle stage IV-VI) and 5 control cases (neurologically normal, CERAD no AD, Braak 0-II) with available *APOE* genotypes, were selected from each centre. Details regarding the composition of the cohorts received from each centre, including mean age, age range and standard deviation are provided in **Table 4**. Paraffin sections (7 μm) were cut from the frontal, temporal, parietal and occipital lobes blocks for all cases, and were sent to Bristol for staining and immunolabelling. Sections from Bristol were cut from large brain blocks and mounted on glass slides measuring 3 x 1.5 inches. Sections from Oxford and CFAS cohorts were cut from smaller blocks and mounted on 3 x 1 inch glass slides; whilst the blocks were smaller than those from Bristol; the sections nonetheless largely filled the area of the slide adjacent to the label.

For CAA staining, approximately 300 sections (one each from the frontal, temporal, parietal and occipital lobes for each of the cases studied) were immunolabelled with 4G8 antibody to Aβ as described above. For assessment of vasculopathy, sections from all of the blocks were also stained with H&E. All of the stained sections from each centre were anonymised in terms of diagnosis and circulated to the participating neuropathologists (ME, PI, SL) for assessment of CAA and vasculopathy according to the agreed protocol that is summarised in **Table 3**. Under x10 objective the whole section was scanned and allocated a score for CAA severity that best fitted the description in **Table 3**. More detailed descriptions of the type of vasculopathy (thrombosis, micro-haemorrhage, concentric splitting of vessel wall, or fibrinoid necrosis) were recorded for future reference. **Figures 2-4** provide illustrative examples of arteriolar CAA of varying severity, capillary CAA and examples of vasculopathy. Scores were sent to Bristol for collation and then externally to FM and TM for statistical analysis independent of the project co-ordinators. To determine the prevalence of parenchymal, meningeal and capillary CAA and vasculopathy the median score for each of the four brain regions was assessed for each case. The median score of the 3 assessors for each case was also calculated for all CAA types and vasculopathy.

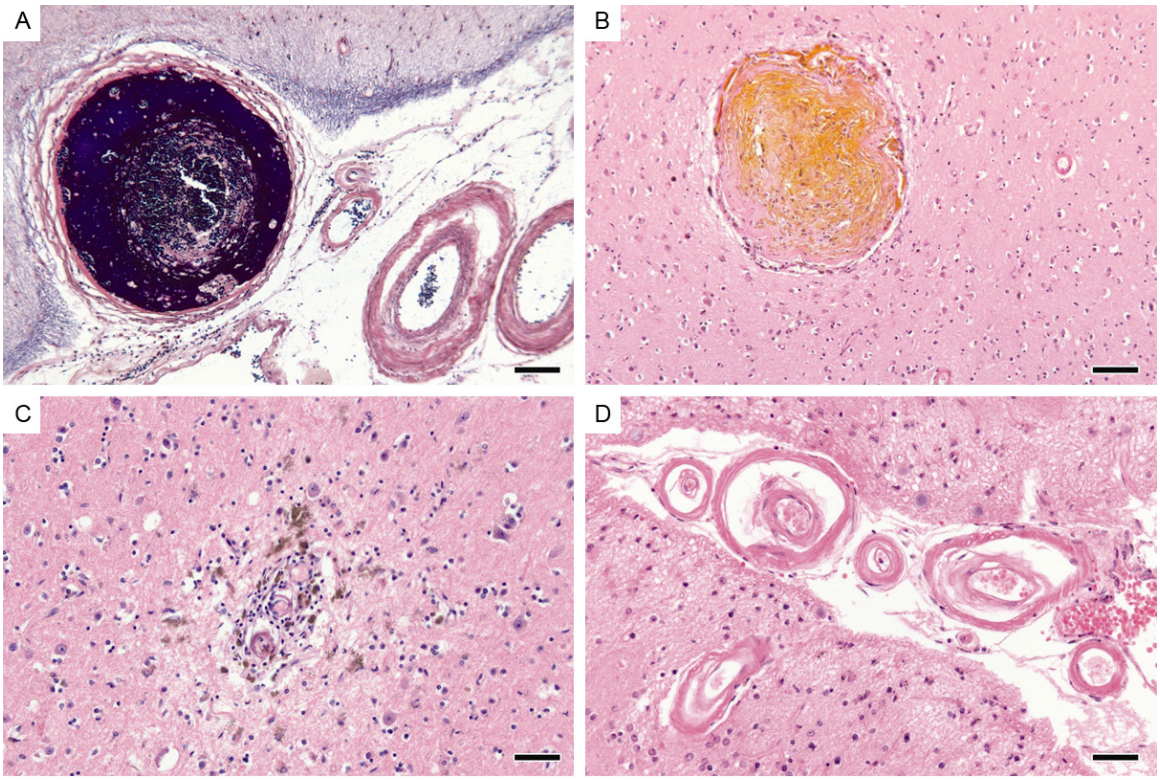

Figure 4. Examples of CAA-associated vasculopathy. A. Fibrinoid necrosis, confirmed here by staining with phosphotungstic acid/haematoxylin. B. Thrombosis of a vessel shown in an adjacent section to contain Aβ. C. Microhaemorrhage adjacent to a small amyloid-laden arteriole. D. Concentric splitting of the wall of amyloid-laden vessels ('double-barrelling'). Sections are scored as zero if there is no vasculopathy, 1 if only occasional vessels are affected and 2 if many vessels are affected. The scale bars represent 100 μm in A and B, and 50 μm in C and D.

Statistical analysis

Inter-rater reliability: The extent of agreement between the three raters in relation to: severity of parenchymal, meningeal and capillary CAA as well as vasculopathy, in the four different brain areas, was assessed by calculating Gwet's AC2 coefficients. The choice of this method was based on the fact that this is a paradox-resistant alternative to Kappa's coefficient when the overall percentage agreement is high [31]. The coefficient calculations were performed using Agreestat 2011.2 programme (Advanced Analytics, Gaithersburg, MD, USA). An AC2 coefficient of zero suggested that any agreement was due to chance. The extent of agreement was assessed using the benchmark proposed by Landis and Koch [32], a coefficient >0.6 indicating substantial agreement and a value >0.8 near-perfect agreement. A p value <0.05 suggested that agreement was not due to chance.

Relationships between APOE, CAA and vasculopathy: The effect of the number of *APOE* ε2 and ε4 alleles on the occurrence of AD was assessed by Fisher's exact test. The effects of *APOE* on CAA and vasculopathy were investigated by a weighted cross-tabulation for multiple readers, design-based F test, and then further quantified by weighted logistic, ordered logistic regression adjusted for cohort source and case-control status. The presence of *APOE* alleles (ε2 and ε4) was adjusted (for each other) within the analysis. Results presented are the odds of the *APOE* ε2 or ε4 allele increasing the likelihood of CAA and vasculopathy. A 95% confidence interval was calculated for the odds ratio (OR). A p value <0.05 was regarded as statistically significant.

Results

Agreement of protocol

The aim of this study was to develop a protocol for the assessment of CAA severity in post-mortem tissue by collating opinions from experts in the CAA field. This was achieved using a Delphi-style questionnaire methodology. The most

Table 5. Inter-rater reliability on the neuropathological burden according to brain regions

Brain Areas	Neuropathological burden	Gwet's AC2 coefficient	Standard Error	95% CI (AC2)	*p*-value***
All	pCAA	0.857	0.050	0.758 to 0.957	<0.001
	mCAA	0.886	0.042	0.801 to 0.971	<0.001
	capCAA	0.875	0.047	0.782 to 0.969	<0.001
	vasculopathy	0.969	0.019	0.93 to 1.000	<0.001
Frontal	pCAA	0.823	0.040	0.743 to 0.902	<0.001
	mCAA	0.853	0.041	0.771 to 0.935	<0.001
	capCAA	0.939	0.032	0.876 to 1.000	<0.001
	vasculopathy	0.959	0.035	0.889 to 1.000	<0.001
Temporal	pCAA	0.776	0.071	0.635 to 0.917	<0.001
	mCAA	0.887	0.056	0.774 to 0.999	<0.001
	capCAA	0.810	0.077	0.656 to 0.964	<0.001
	vasculopathy	0.931	0.034	0.862 to 0.999	<0.001
Parietal	pCAA	0.865	0.032	0.802 to 0.929	<0.001
	mCAA	0.882	0.040	0.803 to 0.961	<0.001
	capCAA	0.902	0.035	0.832 to 0.972	<0.001
	vasculopathy	0.993	0.006	0.982 to 1.000	<0.001
Occipital	pCAA	0.785	0.058	0.670 to 0.901	<0.001
	mCAA	0.849	0.068	0.714 to 0.984	<0.001
	capCAA	0.638	0.086	0.467 to 0.808	<0.001
	vasculopathy	0.972	0.020	0.932 to 1.000	<0.001

Significance at ***p<0.001. pCAA: parenchymal CAA, mCAA: meningeal CAA, capCAA: capillary CAA.

prevalently suggested objectives by participants were integrated to form a protocol that was then put forward for validation. The key objectives were to score parenchymal and meningeal CAA separately; to record individual regional scores; to identify capillary CAA and to record secondary pathologies such as fibrinoid necrosis, all in a number of regions of cortex to include the occipital lobe (**Table 2**).

These objectives were incorporated into a protocol (**Table 3**) that involved scoring parenchymal and meningeal CAA individually on a 0-3 scale, capillary CAA as present/absent and vasculopathy on a 0-2 scale in designated Brodmann areas from the frontal, temporal, parietal and occipital lobes of a cohort of brains from three collaborating centres (**Table 4**).

Outcome of validation exercise

Table 5 shows Gwet's AC2 coefficients for parenchymal, meningeal and capillary CAA and vasculopathy for each individual brain region assessed and all brain regions combined. In general all levels of severity of CAA, presence or absence of capillary CAA and presence or absence of vasculopathy were seen to have AC2 coefficients greater than 0.8, implying near-perfect agreement. The only exceptions were for parenchymal CAA in the temporal lobe (AC2=0.776) and parenchymal (0.785) and capillary CAA (0.638) in the occipital lobes, where agreement was nonetheless substantial. Importantly all variables had *p* values <0.001 indicating that agreement was not due to chance.

Having achieved near perfect agreement in most assessments of parenchymal, meningeal and capillary CAA and vasculopathy, we determined whether relationships previously reported between CAA, vasculopathy and *APOE* genotype applied to the data obtained by the three independent raters in our validation study. The analysis was performed on a final cohort of 51 AD and 14 control cases for which *APOE* genotype data was available.

APOE as a risk factor for AD

Before assessing relationships with CAA and vasculopathy we firstly looked to confirm the established relationship between the *APOE* ε4 allele and the occurrence of AD [33] (**Table 6**). The *APOE* ε4 allele frequency was higher in AD cases and the number of ε4 homozygotes was also greater than in controls. The cumulative

Table 6. *APOE* allele frequencies in AD and control cases

Diagnosis	Centre	*APOE* alleles			Number of ε4 alleles			Number of ε2 alleles		
		ε2	ε3	ε4	0	1	2	0	1	2
AD	Bristol	0.025	0.625	0.350	0.500	0.300	0.200	0.950	0.050	0.000
n=51	Oxford	0.075	0.500	0.425	0.400	0.350	0.250	0.850	0.150	0.000
	CFAS	0.000	0.727	0.273	0.545	0.364	0.091	1.000	0.000	0.000
	Cumulative	0.039	0.598	0.363	0.471	0.338	0.153	0.922	0.078	0.000
Control	Bristol	0.200	0.700	0.100	0.800	0.200	0.000	0.800	0.000	0.200
n=14	Oxford	0.100	0.700	0.200	0.600	0.400	0.000	0.800	0.200	0.000
	CFAS	0.375	0.375	0.250	0.500	0.500	0.000	0.500	0.250	0.250
	Cumulative	0.214	0.607	0.179	0.643	0.357	0.000	0.714	0.142	0.142
p (Fisher's exact test)		0.007	1.000	0.072		0.192			0.032	

Table 7. Relationship between *APOE* ε4, CAA and vasculopathy in control and AD brains

		Unadj p	OR	95% CI (OR)	p
All	pCAA	0.030	1.79	(1.00-3.21)	0.050
	mCAA	<0.001	1.69	(0.92-3.10)	0.089
	capCAA	0.176	1.55	(0.68-3.50)	0.293
	vasculopathy	0.967	0.66	(0.28-1.54)	0.337
Frontal	pCAA	0.204	1.73	(0.99-3.02)	0.056
	mCAA	<0.001	2.29	(1.28-4.12)	0.006**
	capCAA	0.936	0.94	(0.32-2.75)	0.916
	vasculopathy	0.688	0.59	(0.21-1.64)	0.310
Temporal	pCAA	0.130	1.27	(0.71-2.30)	0.421
	mCAA	0.045	1.37	(0.78-2.40)	0.271
	capCAA	0.606	1.33	(0.53-3.35)	0.547
	vasculopathy	0.159	0.41	(0.14-1.22)	0.109
Parietal	pCAA	0.182	1.27	(0.71-2.28)	0.418
	mCAA	0.000	1.32	(0.73-2.37)	0.356
	capCAA	0.708	0.87	(0.34-2.25)	0.779
	vasculopathy	0.589	0.75	(0.27-2.05)	0.572
Occipital	pCAA	0.001	2.79	(1.54-5.04)	0.001***
	mCAA	0.012	1.87	(1.01-3.49)	0.048*
	capCAA	<0.001	3.60	(1.88-6.90)	<0.001***
	vasculopathy	0.024	2.61	(1.19-5.71)	0.017*

Significance at *p<0.05, significance at **p<0.01, significance at ***p<0.001.

frequency of the *APOE* ε2 allele was clearly elevated in controls compared to AD cases (Fisher's exact test, p=0.030). When comparing the cumulative frequency between cohorts, there were no significant differences in the distribution of the number of the *APOE* ε2 alleles among controls (p=0.667) or among AD (p= 0.521), no difference was noticed also in the distribution of the number of the *APOE* ε4 alleles (controls, p=0.800; AD, p=0.856) according to Fisher's exact test.

CAA prevalence

Non-demented elderly controls: Across all four regions investigated (frontal, temporal, parietal and occipital lobes), CAA was evident in 78.5-85.7% of cases: 50.0-64.3% of all cases had parenchymal CAA, 78.5-85.7% had meningeal CAA and 7.1-14.3% had capillary CAA.

CAA was most prevalent in the occipital lobe 85.7% followed by the temporal (78.6%), parietal (71.4%) and frontal (64-71.4%) lobes. Capillary CAA was considerably more abundant in the occipital lobe (21.4%) than the frontal (7.1%), parietal (7%) and temporal (0-7.1%) lobes.

AD cases: CAA was found in 82.4-94.1% of the cases assessed: 66.680.4% of all cases had parenchymal CAA, 80.4-92.2% meningeal CAA and 7.8-23.5% capillary CAA.

The most affected region was the occipital lobe, in which 92.2-100.0% of cases had some evidence of arteriolar CAA. The parietal lobe was least affected (76.5-80.4%). Capillary CAA was most commonly observed in the occipital lobe (35.3-47.1%), followed by the temporal (7.8-19.6%), parietal (7.8-17.7% and frontal (5.9-11.8%) lobes.

APOE as a risk factor for CAA

We investigated the association between *APOE* genotype and the presence and distribution of

Table 8. Relationship between *APOE* ε2, CAA and vasculopathy in control and AD brains

		Unadj p	OR	95% CI (OR)	p
All	pCAA	0.001	4.38	(2.45-7.80)	<0.001***
	mCAA	0.001	10.93	(4.33-27.57)	<0.001***
	capCAA	0.160	2.91	(1.10-7.66)	0.031*
	vasculopathy	0.255	0.52	(0.13-2.09)	0.357
Frontal	pCAA	0.019	3.93	(2.11-7.30)	<0.001***
	mCAA	0.010	5.74	(2.89-11.38)	<0.001***
	capCAA	0.288	1.69	(0.55-5.24)	0.361
	vasculopathy	0.359	0.37	(0.05-3.02)	0.352
Temporal	pCAA	<0.001	5.11	(2.64-9.89)	<0.001***
	mCAA	0.001	9.88	(3.56-27.43)	<0.001***
	capCAA	0.504	2.71	(0.77-9.60)	0.121
	vasculopathy	0.556	2.20	(0.56-8.68)	0.257
Parietal	pCAA	0.002	3.28	(1.77-6.08)	<0.001***
	mCAA	<0.001	6.56	(2.32-18.59)	<0.001***
	capCAA	0.004	5.69	(1.70-18.99)	0.005**
	vasculopathy	0.649	0.40	(0.05-3.09)	0.376
Occipital	pCAA	0.001	3.15	(1.49-6.68)	0.003**
	mCAA	0.007	7.16	(2.31-22.13)	0.001***
	capCAA	0.381	1.23	(0.41-3.70)	0.714
	vasculopathy	0.152	1.48	(0.27-8.24)	0.654

Significance at *p<0.05, significance at **p<0.01, significance at ***p<0.001.

CAA (**Tables 7**-**10**). When AD and non-demented elderly controls were combined (**Table 7**), the association between CAA and *APOE* ε4 was strong in the frontal lobe, where ε4 was associated with meningeal CAA, and in the occipital lobe, where ε4 was associated with parenchymal, meningeal and capillary CAA and vasculopathy. However possession of *APOE* ε2 was associated with a considerably increased likelihood of CAA in AD and controls combined (**Table 8**), especially so for parenchymal and meningeal CAA and capillary CAA in the parietal lobe and 4 regions combined.

When we looked at the non-demented elderly controls separately (**Table 9**), *APOE* ε2 was strongly associated with CAA in both the parenchyma and meninges in all four regions separately and combined. Conversely, *APOE* ε4 was significantly associated only with parenchymal CAA in the occipital lobe. Only one control case had capillary CAA and very few had any vasculopathy.

In AD the association of the *APOE* ε2 allele with CAA was strong in both the parenchyma and meninges in all four regions both separately and combined (**Table 10**) except for the occipital parenchymal CAA. In AD the association between meningeal CAA and *APOE* ε4 allele was strong in the four areas combined, frontal and occipital lobes, and parenchymal CAA in the occipital lobe were associated with *APOE* ε4.

Relationship between APOE genotype and CAA type

Tables 11A, **11B** summarise the relationship between *APOE* genotype and the different types of CAA in our series. All individuals with the *APOE* ε2/2 genotype had CAA-Type 2 (i.e. Aβ deposition restricted to arterial vessels). In the uncontrolled weighted analysis, the odds ratio for CAA-Type 1 (i.e. characterised by the presence of capillary Aβ with or without Aβ deposition in arterial vessels) versus non-CAA-Type 1 for the presence of *APOE* ε4/4 genotype was 10.6 (95% CI 3.8; 29.3). When we controlled the analyses for cohort and diagnosis, this relationship was still very strong (OR 8.0 95% CI 2.8; 23.3).

Discussion

Developed criteria

The aim of this study was to develop novel criteria for scoring the severity of CAA and diagnosing concomitant vasculopathy, in a standardised, reproducible and biologically meaningful way. The reliability of the criteria was verified by three experienced neuropathologists (ME, PI and SL), whose assessment of CAA and vasculopathy in a cohort of AD cases and non-demented elderly controls from 3 centres was analysed using Gwet's AC2 statistic, a new statistical method designed to allow the calculation of agreement in situations where there is high agreement of a rare event such as that of the presence of capillary CAA or vasculopathy [31]. When we applied the arbitrary boundaries of Landis and Koch [32], our coefficients were found, in the main, to be outstanding (near-perfect) for arteriolar and capillary CAA and vasculopathy in the four brain regions assessed. This was particularly encouraging for parenchymal

Table 9. Relationship between *APOE* ε2 and ε4 and CAA in control brains

		APOE ε2			*APOE* ε4		
		OR	95% CI (OR)	p	OR	95% CI (OR)	p
All	pCAA	17.09	(3.25-89.90)	0.001***	1.64	(0.48-5.64)	0.425
	mCAA	38.73	(8.67-173.12)	<0.001***	0.53	(0.11-2.64)	0.432
Frontal	pCAA	6.47	(1.13-37.07)	0.037*	2.99	(0.91-9.86)	0.071
	mCAA	7.18	(1.20-42.90)	0.032*	2.21	(0.59-8.21)	0.229
Temporal	pCAA	11.15	(2.51-49.61)	0.002**	0.49	(0.14-1.76)	0.266
	mCAA	23.05	(5.30-100.18)	<0.001***	0.38	(0.10-1.39)	0.138
Parietal	pCAA	6.31	(1.43-27.87)	0.016*	0.85	(0.23-3.10)	0.798
	mCAA	19.21	(4.61-80.14)	<0.001***	0.66	(0.17-2.60)	0.549
Occipital	pCAA	11.09	(2.04-60.29)	0.006**	4.56	(1.05-19.79)	0.043*
	mCAA	8.87	(2.11-37.25)	0.004**	1.03	(0.20-5.34)	0.969

There were not enough cases with capillary CAA or vasculopathy for statistical analysis. Significance at *p<0.05, significance at **p<0.01, significance at ***p<0.001.

Table 10. Relationship between *APOE* ε2 and ε4 and CAA in AD brains

		APOE ε2			*APOE* ε4		
		OR	95% CI (OR)	p	OR	95% CI (OR)	p
All	pCAA	2.60	(1.35-5.03)	0.005**	1.71	(0.90-3.27)	0.102
	mCAA	8.03	(2.77-23.27)	<0.001**	2.14	(1.09-4.21)	0.028*
Frontal	pCAA	2.56	(1.22-5.36)	0.013*	1.44	(0.78-2.67)	0.243
	mCAA	6.03	(2.14-16.96)	0.001***	2.37	(1.21-4.65)	0.012*
Temporal	pCAA	3.80	(1.59-9.08)	0.003**	1.43	(0.73-2.81)	0.295
	mCAA	6.67	(1.75-25.39)	0.006**	1.78	(0.95-3.33)	0.069
Parietal	pCAA	2.50	(1.25-5.00)	0.010*	1.38	(0.72-2.62)	0.328
	mCAA	4.74	(1.42-15.78)	0.012*	1.65	(0.85-3.18)	0.135
Occipital	pCAA	2.18	(0.74-6.47)	0.159	2.55	(1.35-4.83)	0.004**
	mCAA	6.27	(1.13-34.66)	0.036*	2.18	(1.11-4.27)	0.024*

There were not enough cases with capillary CAA or vasculopathy for statistical analysis. Significance at *p<0.05, significance at **p<0.01, significance at ***p<0.001.

Table 11A. Weighted percentage of individuals with each *APOE* genotype among each CAA type (row percentages)

	22	23	24	33	34	44
Type 0	0.0	0.0	0.0	58.1	41.9	0.0
Type 1	0.0	9.0	3.8	25.6	29.5	32.0
Type 2	7.0	5.8	3.5	50.0	27.9	5.8

and meningeal forms of CAA that show very variable severity and might therefore have been expected to generate low AC2 coefficients. The lowest coefficient in our study was for capillary CAA in the occipital lobe. Although the agreement was still considered substantial, the lower AC2 coefficient probably reflects the greater difficulty in identifying capillary CAA, particularly in the context of abundant plaque pathology. The recognition of capillary CAA as a discrete pathological process is relatively recent and the greater experience of the neuropathologists in identifying arteriolar CAA may also have contributed to the slightly different levels of consistency in identifying the two processes.

The AC2 coefficient was near-perfect for vasculopathy. Whilst this is an encouraging finding, in our AD cohort the occurrence of vasculopathy was relatively rare, more so than that of capillary CAA. In part the high coefficient reflects the agreement amongst the assessors that there was no vasculopathy in the majority of cases. The robustness of identifying vasculopathy should perhaps be evaluated in a series of cases with CAA-associated haemorrhage.

Application of criteria

Prevalence: CAA was previously reported to be present in between 90-100% of AD cases and in approximately 30% of non-demented controls [1, 26, 34-37]. In this study we found CAA to be present in 80-92% of AD cases and 79-86% of controls. The seemingly inflated occurrence of CAA in control brains is likely to be due to a number of methodological issues. The first of these was the inclusion of the occipital lobe, which is the most commonly affected part of the brain but which was not assessed in most previous studies [38]. Another contributor may be the sampling method for CAA, which in the current protocol involves scanning the

Table 11B. Weighted percentage of individuals with each CAA type among each genotype (column percentages)

	22	23	24	33	34	44
Type 0	0.0	0.0	0.0	22.2	21.7	0.0
Type 1	0.0	58.3	50.0	24.7	38.3	83.3
Type 2	100	41.7	50.0	53.1	40.0	16.7

whole section rather than assessing a specific number of randomly selected fields only. Lastly, the use of the 4G8 antibody may be relevant as it seems to be more effective than some other Aβ antibodies at detecting CAA. Clearly it would be of benefit to apply this protocol to a larger series of post-mortem brain tissue samples from non-demented elderly to determine whether our findings can be replicated.

APOE, CAA and vasculopathy

The present study suggests that *APOE* ε2 may have a more important role in the development of arteriolar CAA than has been previously appreciated. This is particularly so in the absence of AD - in control brains possession of *APOE* ε2 was a strong risk factor for the development of arteriolar CAA in all brain regions assessed. Possession of *APOE* ε4 seemed to raise the risk of CAA mainly in the occipital lobe and predominantly in patients with AD who had both arteriolar and capillary accumulation of Aβ.

Conclusions

We have developed an assessment protocol for CAA that can be reliably used to assess the presence and/or severity of parenchymal, meningeal and capillary CAA, and CAA-associated vasculopathy, in post-mortem brain tissue. The novel identification of significant association between *APOE* ε2 and CAA in control brains highlights the value of the assessment protocol and its utility in promoting collaboration and pooling of data between centres.

Acknowledgements

We should like to thank Shane McParland and Nicky Price for their technical assistance, Drs Matthew Frosch and Catharine Joachim for their contributions throughout the rounds of the protocol development and also Professors James Nicoll and Dietmar Thal for their advice in the later stages of the protocol development.

This study was supported by grants from ARUK (Alzheimer's Research UK) and BRACE (Bristol Research into Alzheimer's and Care of the Elderly).

Disclosure of conflict of interest

No conflicts of interest to be declared.

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References

[1] Love S, Nicoll JA, Hughes A and Wilcock GK. APOE and cerebral amyloid angiopathy in the elderly. Neuroreport 2003; 14: 1535-1536.

[2] Love S, Miners S, Palmer J, Chalmers K and Kehoe P. Insights into the pathogenesis and pathogenicity of cerebral amyloid angiopathy. Front Biosci 2009; 14: 4778-4792.

[3] Keage HA, Carare RO, Friedland RP, Ince PG, Love S, Nicoll JA, Wharton SB, Weller RO and Brayne C. Population studies of sporadic cerebral amyloid angiopathy and dementia: a systematic review. BMC Neurol 2009; 9: 3.

[4] Greenberg SM, Rebeck GW, Vonsattel JP, Gomez-Isla T and Hyman BT. Apolipoprotein E epsilon 4 and cerebral hemorrhage associated with amyloid angiopathy. Ann Neurol 1995; 38: 254-259.

[5] Nicoll JA, Burnett C, Love S, Graham DI, Dewar D, Ironside JW, Stewart J and Vinters HV. High frequency of apolipoprotein E epsilon 2 allele in hemorrhage due to cerebral amyloid angiopathy. Ann Neurol 1997; 41: 716-721.

[6] Cadavid D, Mena H, Koeller K and Frommelt RA. Cerebral beta amyloid angiopathy is a risk factor for cerebral ischemic infarction. A case control study in human brain biopsies. J Neuropathol Exp Neurol 2000; 59: 768-773.

[7] Olichney JM, Hansen LA, Hofstetter CR, Lee JH, Katzman R and Thal LJ. Association between severe cerebral amyloid angiopathy and cerebrovascular lesions in Alzheimer disease is not a spurious one attributable to apolipoprotein E4. Arch Neurol 2000; 57: 869-874.

[8] Vinters HV. Cerebral amyloid angiopathy. A critical review. Stroke 1987; 18: 311-324.

[9] Weller RO, Preston SD, Subash M and Carare RO. Cerebral amyloid angiopathy in the aetiology and immunotherapy of Alzheimer disease. Alzheimers Res Ther 2009; 1: 6.

[10] Herzig MC, Van Nostrand WE and Jucker M. Mechanism of cerebral beta-amyloid angiopa-

thy: murine and cellular models. Brain Pathol 2006; 16: 40-54.
[11] Miners JS, Kehoe P and Love S. Neprilysin protects against cerebral amyloid angiopathy and Abeta-induced degeneration of cerebrovascular smooth muscle cells. Brain Pathol 2011; 21: 594-605.
[12] Pahnke J, Wolkenhauer O, Krohn M and Walker LC. Clinico-pathologic function of cerebral ABC transporters - implications for the pathogenesis of Alzheimer's disease. Curr Alzheimer Res 2008; 5: 396-405.
[13] Weller RO, Subash M, Preston SD, Mazanti I and Carare RO. Perivascular drainage of amyloid-beta peptides from the brain and its failure in cerebral amyloid angiopathy and Alzheimer's disease. Brain Pathol 2008; 18: 253-266.
[14] Fryer JD, Simmons K, Parsadanian M, Bales KR, Paul SM, Sullivan PM and Holtzman DM. Human apolipoprotein E4 alters the amyloid-beta 40:42 ratio and promotes the formation of cerebral amyloid angiopathy in an amyloid precursor protein transgenic model. J Neurosci 2005; 25: 2803-2810.
[15] Miners JS, Van Helmond Z, Chalmers K, Wilcock G, Love S and Kehoe PG. Decreased expression and activity of neprilysin in Alzheimer disease are associated with cerebral amyloid angiopathy. J Neuropathol Exp Neurol 2006; 65: 1012-1021.
[16] Thal DR, Ghebremedhin E, Rub U, Yamaguchi H, Del Tredici K and Braak H. Two types of sporadic cerebral amyloid angiopathy. J Neuropathol Exp Neurol 2002; 61: 282-293.
[17] Thal DR, Papassotiropoulos A, Saido TC, Griffin WS, Mrak RE, Kolsch H, Del Tredici K, Attems J and Ghebremedhin E. Capillary cerebral amyloid angiopathy identifies a distinct APOE epsilon4-associated subtype of sporadic Alzheimer's disease. Acta Neuropathol 2010; 120: 169-183.
[18] Premkumar DR, Cohen DL, Hedera P, Friedland RP and Kalaria RN. Apolipoprotein E-epsilon4 alleles in cerebral amyloid angiopathy and cerebrovascular pathology associated with Alzheimer's disease. Am J Pathol 1996; 148: 2083-2095.
[19] Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D and Roses AD. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. Proc Natl Acad Sci U S A 1993; 90: 9649-9653.
[20] Heinonen O, Lehtovirta M, Soininen H, Helisalmi S, Mannermaa A, Sorvari H, Kosunen O, Paljarvi L, Ryynanen M and Riekkinen PJ Sr. Alzheimer pathology of patients carrying apolipoprotein E epsilon 4 allele. Neurobiol Aging 1995; 16: 505-513.
[21] Olichney JM, Hansen LA, Galasko D, Saitoh T, Hofstetter CR, Katzman R and Thal LJ. The apolipoprotein E epsilon 4 allele is associated with increased neuritic plaques and cerebral amyloid angiopathy in Alzheimer's disease and Lewy body variant. Neurology 1996; 47: 190-196.
[22] Rannikmae K, Kalaria RN, Greenberg SM, Chui HC, Schmitt FA, Samarasekera N, Al-Shahi Salman R and Sudlow CL. APOE associations with severe CAA-associated vasculopathic changes: collaborative meta-analysis. J Neurol Neurosurg Psychiatry 2014; 85: 300-5.
[23] Rannikmae K, Samarasekera N, Martinez-Gonzalez NA, Al-Shahi Salman R and Sudlow CL. Genetics of cerebral amyloid angiopathy: systematic review and meta-analysis. J Neurol Neurosurg Psychiatry 2013; 84: 901-908.
[24] Greenberg S, Vonsattel J, Segal A, Chiu R, Clatworthy A, Liao A, Hyman B and Rebeck G. Association of apolipoprotein E epsilon2 and vasculopathy in cerebral amyloid angiopathy. Neurology 1998; 50: 961-965.
[25] Olichney JM, Hansen LA, Hofstetter CR, Grundman M, Katzman R and Thal LJ. Cerebral infarction in Alzheimer's disease is associated with severe amyloid angiopathy and hypertension. Arch Neurol 1995; 52: 702-708.
[26] Vinters HV and Gilbert JJ. Cerebral amyloid angiopathy: incidence and complications in the aging brain. II. The distribution of amyloid vascular changes. Stroke 1983; 14: 924-928.
[27] Vonsattel JP, Myers RH, Hedley-Whyte ET, Ropper AH, Bird ED and Richardson EP Jr. Cerebral amyloid angiopathy without and with cerebral hemorrhages: a comparative histological study. Ann Neurol 1991; 30: 637-649.
[28] Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G and Berg L. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991; 41: 479-486.
[29] Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, Jorm A, Mathers C, Menezes PR, Rimmer E, Scazufca M; Alzheimer's Disease International. Global prevalence of dementia: a Delphi consensus study. Lancet 2005; 366: 2112-2117.
[30] Linstone HA and Turoff M. The Delphi method: techniques and applications. Addison-Wesley Pub. Co., Advanced Book Program, 1975.
[31] Gwet KL. Computing inter-rater reliability and its variance in the presence of high agreement. Br J Math Stat Psychol 2008; 61: 29-48.

[32] Landis JR and Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977; 33: 159-174.
[33] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL and Pericak-Vance MA. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993; 261: 921-923.
[34] Esiri MM and Wilcock GK. Cerebral amyloid angiopathy in dementia and old age. J Neurol Neurosurg Psychiatry 1986; 49: 1221-1226.
[35] Masuda J, Tanaka K, Ueda K and Omae T. Autopsy study of incidence and distribution of cerebral amyloid angiopathy in Hisayama, Japan. Stroke 1988; 19: 205-210.
[36] Tomonaga M. Cerebral amyloid angiopathy in the elderly. J Am Geriatr Soc 1981; 29: 151-157.
[37] Yamada M, Tsukagoshi H, Otomo E and Hayakawa M. Cerebral amyloid angiopathy in the aged. J Neurol 1987; 234: 371-376.
[38] Attems J, Jellinger KA and Lintner F. Alzheimer's disease pathology influences severity and topographical distribution of cerebral amyloid angiopathy. Acta Neuropathol 2005; 110: 222-231.