No apparent association between beta-amyloid deposition and apolipoprotein E genotype in the non-demented aging brain in a Malaysian population

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Abstract

Little is known about the relationship between cerebral beta amyloid (Aβ) deposition and apolipoprotein E (ApoE) genotype in either Alzheimer disease or the aging brain in multi-ethnic Southeast Asia. We prospectively examined Aβ deposition in relation to ApoE genotype in 50 normal, non-demented, aging brains drawn from a Malaysian population, aged 52-92 years, using immunohistochemistry to detect Aβ and direct PCR sequencing for genotyping. The prevalence of Aβ deposition was 8%. There was no apparent association between Aβ deposition and possession of the ApoE ε4 allele in our cohort. Out of 4 cases with Aβ deposition, only one case was heterozygous for the ε4 allele; 3 cases did not have the ε4 allele. The Aβ deposition appears to increase with age and is more likely to be vascular-type deposition (cerebral amyloid angiopathy) rather than parenchymal deposition in the form of diffuse and neuritic plaques. A larger sample size with more cases of ApoE ε4 and Aβ deposition is needed to provide conclusive evidence for the apparent non-association between ApoE ε4 and Aβ deposition in the aging brain in our multi-ethnic local population.

INTRODUCTION

Aging and possession of apolipoprotein E (ApoE) ε4 allele are important risk factors for Alzheimer disease (AD). The ApoE ε4 allele has been suggested to hasten AD onset by modulating the deposition and clearance of beta-amyloid (Aβ), a 39-43 amino acid protein aggregate which is the neuropathological hallmark of AD.[1]

ApoE is a plasma protein that plays an important role in transporting and distributing lipids among tissues. There are three human ApoE isoforms, i.e. ApoE2, ApoE3 and ApoE4, which are encoded by three co-dominant alleles (ε2, ε3, and ε4 respectively) at a single gene locus on chromosome 19q13.2. This results in six possible genotypes as each individual possesses two allelic copies of a gene, i.e. E2E2, E3E3, E4E4, in homozygotes and E2E3, E2E4 and E3E4, in heterozygotes. The genotypes differ in having cysteine (Cys) and arginine (Arg) at positions 112 and 158.[2] For example, E2E2 has both Cys at positions 112 and 158; E3E3 has Cys at position 112 and Arg at position 158, whereas E4E4 has both Arg at positions 112 and 158.

Several studies from Europe, America and Japan have shown that possession of ApoE ε4 allele promotes Aβ deposition in non-demented, 'normal' aging elderly populations.[3-7] However, in multi-ethnic Southeast Asia, where the incidence of AD is lower than developed countries as reviewed in the World Alzheimer Report 2009[8], little is known about the relationship between the cerebral Aβ deposition and ApoE genotype. Hence, in this study we sought to examine the association between ApoE ε4 allele and Aβ deposition in an aging multi-ethnic Malaysian population.

METHODS

Autopsy brains

Whole brains were prospectively collected from subjects > 50 years old who had full autopsies performed in the University of Malaya Medical Centre (UMMC), Malaysia from 1996 to 2000. The causes of death included asphyxia, aspiration pneumonia, hanging, hypovolaemic shock from intestine bleeding, kerosene ingestion, haemopericardium and traumatic injuries. Postmortem delay was approximately 12-24

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hours. None of the collected brains has apparent clinical or gross pathological evidence of AD. The brain was immersion-fixed in 10% (v/v) buffered formalin for at least three weeks before being coronally-sliced into standard 1cm slices. A total of seven representative blocks from various brain regions were obtained from each case and submitted for processing and paraffin embedding. These blocks included: 1) frontal lobe (superior frontal gyrus/middle frontal gyrus); 2) mid-hippocampus (including tail of caudate nucleus and lateral geniculate body); 3) putamen, globus pallidus (two segments), basal nucleus of Meynert and insular cortex; 4) amygdala (including basal nucleus of Meynert, pallidum, hypothalamus and mammillary body); 5) rostral midbrain (substantia nigra, cerebral peduncle and red nucleus); 6) temporal lobe (superior and middle temporal gyri) and 7) inferior parietal lobule.

Aβ immunohistochemistry

The Aβ immunohistochemistry (IHC) was performed using strepavidin-biotin-peroxidase technique as described previously[9], with some modifications. After deparaffinization and dehydration, the tissue sections were pretreated with 80% (v/v) formic acid for 10 minutes to retrieve the antigens.[10] The non-specific protein binding sites were blocked with 5% normal rabbit serum in Tris-buffer saline (TBS, 150 mM NaCl, 50 mM Tris, pH 7.6) for 20 minutes at 37°C. Mouse monoclonal antibody (clone 6F/3D, Dako,USA) was used as primary antibody at 1:100 dilution and incubated for 2 hours at 37°C. The secondary antibody was biotinylated rabbit anti-mouse (Dako, USA) diluted at 1:300, and incubated at room temperature for 30 minutes. To reveal the immunoreactivity, 0.08% (w/v) 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma, USA) in TBS was added in the presence of 0.045% (v/v) hydrogen peroxide. The sections were then counterstained with Harris haematoxylin, dehydrated in a series of graded ethanols and mounted in organic mounting medium, DPX. Positive control tissue was a brain sample from a case of Alzheimer's disease. For negative controls, sections were also stained without the primary antibody or with normal mouse serum in the place of primary antibody.

Apolipoprotein E genotyping

DNA for ApoE genotyping in each case was extracted from 5-10 sections of five μm thick, paraffin embedded tissue. The sections were first digested completely in 1 mg of Proteinase-K (Sigma, USA) overnight, at 37°C, followed by phenol-chloroform extraction as described by Jackson et al.[11] The two polymorphic codons of ApoE alleles were identified by PCR amplification using primers 5'-CTG GAG GAA CAA CTG ACC CCG GTG- 3' and 5'- GCG CAC CCG CAG CTC CTC GGT GCT- 3' for codon 112, and 5'- CTC GCC TCC CAC CTG CGC AAG CTG CGT- 3' and 5'-CAG GCG CTC GCG GAT GGC GCT GAG- 3' for codon 158. The PCR reaction was performed using HotStarTaq DNA Polymerase (Qiagen, USA) with 'hot start' at 95ºC for 15 minutes, followed by 40 cycles of 60 seconds of denaturation at 94ºC, 90 seconds of annealing at 68ºC, and 90 seconds of extension at 72ºC. After 10 minutes of final extension at 72ºC, the PCR products were kept in 4°C. The PCR products were gel-purified as per manufacturer's instruction (Qiagen, USA) before sending for direct PCR sequencing (Research Bioloabs, Singapore and First Base Sdn Bhd, Malaysia).

RESULTS

The 50 cases in this study comprised three major ethnic groups found in Malaysia: Chinese (54%), Indian (32%) and Malay (14%). The mean age was 70.1 ± 10.6 (mean ± SD), with the youngest being 52 and the oldest 92 years. Males constitute 78% of the subjects (Table 1).

Beta-amyloid IHC staining

Out of the 50 cases, four cases (8%) showed positive IHC staining for Aβ (Table 1 and 2). All four cases showed Aβ deposition, either in the parenchyma (as neuritic plaques, mainly core-containing type) or around blood vessels (perivascular or cerebral amyloid angiopathy) (Table 2 and Figure 1). Overall, the frontal and temporal lobes appeared to be the most frequent regions for Aβ deposition while the rostral midbrain was the least likely region for Aβ deposition. Perivascular Aβ deposition rather than plaque deposition was the most common morphological type in our study (Table 2).

ApoE genotyping

ApoE genotyping by direct PCR sequencing showed that 94 % (47 out of 50) of the subjects carried the E3E3 genotype (Table 1). Only two subjects possessed the E2E4 genotype (4%) in our samples, while the remaining one is an E2E3 carrier. None of the subjects was homozygous for

Table 1: Demography, apolipoprotein (ApoE) genotyping and beta-amyloid (Aβ) immunohistochemistry (IHC) in 50 aging brains

Ethnic Groups	Total Number of Cases (%)	Sex (n)	Age Range (Years)	ApoE genotype (% frequency)	Aβ IHC
Chinese	27 (54%)	Male (20)	52-90	E3E3, 25 cases (92.5%)	-
		Female (7)	75-92	E2E4, 1 case (3.7%)	+ (1 case)
				E2E3, 1 case (3.7%)	+ (1 case)
Indian	16 (32%)	Male (13)	54-90	E3E3, 15 cases (93.8%)	+ (1 case)
		Female (3)	66-84	E2E4, 1 case (6.3%)	-
Malay	7 (14%)	Male (6)	52-75	E3E3, 7 cases (100%)	+ (1 case)
		Female (1)	68		

n = number of cases; - = negative staining; + = positive staining

the ε2 or ε4 allele. Out of the two E4 positive cases, only one case had Aβ deposition, mainly of perivascular-type (Table 2). The other three cases with Aβ deposition did not possess any ε4 allele.
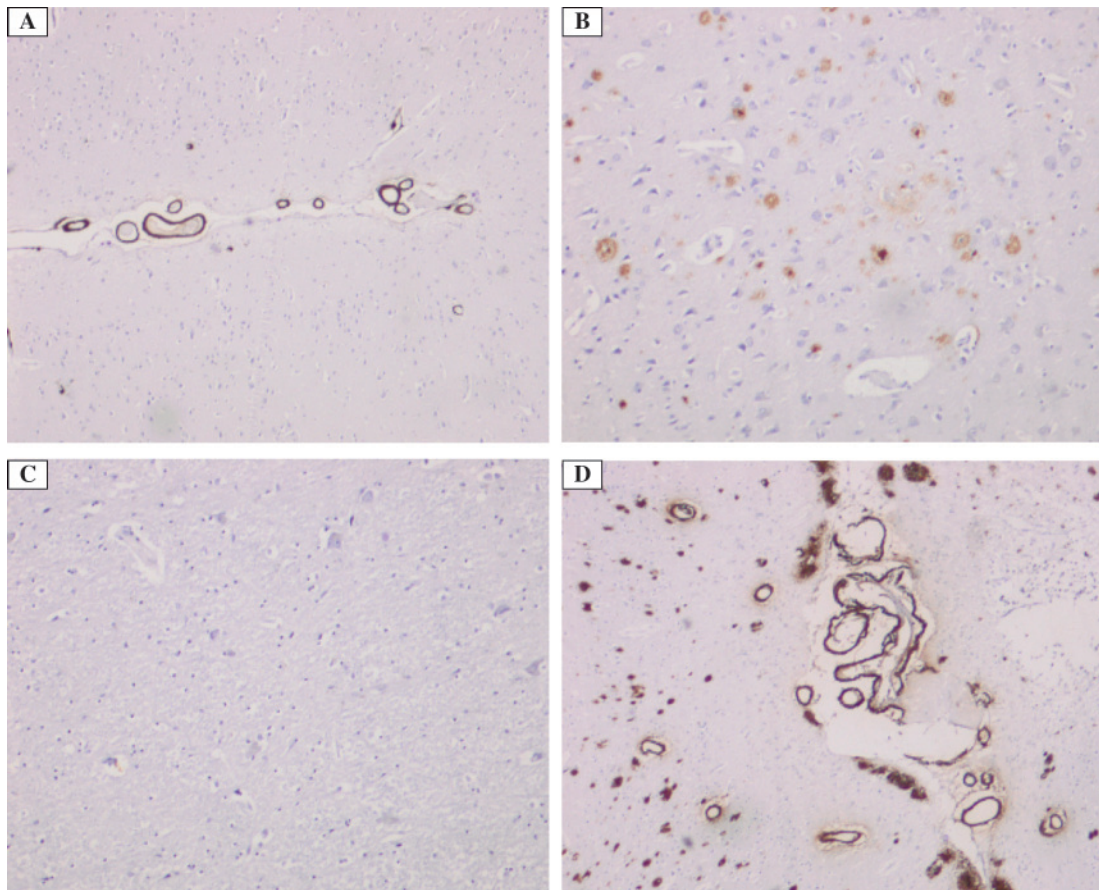
Figure 1: Cerebral perivascular-type (A) and parenchymal plaque (B) deposition of beta-amyloid. C shows a negative control and D is positive control from a case of Alzheimer's disease that has both perivascular and parenchymal amyloid deposits. A, C and D: x4 objective; B: x10 objective. A-D: Immunohistochemistry stain with haematoxylin counter stain.

Table 2: Demography and ApoE genotype in four cases with beta-amyloid deposition.

Case No.	Sex/age	Ethnic group	ApoE genotype	Beta-amyloid immunohistochemistry staining													
				Block 1		Block 2		Block 3		Block 4		Block 5		Block 6		Block 7	
				BV	PP	BV	PP	BV	PP	BV	PP	BV	PP	BV	PP	BV	PP
A93/318	Male/60 years	Indian	E3E3	+	++	+	-	+	+	+	-	-	-	++	++	-	-
A98/266	Male/70 years	Malay	E3E3	++	++	+	++	-	+	-	+	-	+	+	++	-	++
A97/309	Male/81 years	Chinese	E2E3	++	++	+	++	+	+	+	++	-	-	+	+	+	++
A97/145	Female/75 years	Chinese	E2E4	++	-	++	-	++	-	++	+	+	-	++	-	++	-

BV = Blood vessel (perivascular deposition): + = rare/occasional blood vessel positive; ++ = <50% blood vessel positive
PP = Parenchymal plaque: + = rare/occasional plaques (<10 plaques per field, x10 objective); ++ = 11 to 50 plaques per field, x10 objective)

Representative blocks:
Block 1 = Frontal lobe (superior frontal gyrus/middle frontal gyrus)
Block 2 = Mid-hippocampus (including tail of caudate nucleus and lateral geniculate body)
Block 3 = Putamen, globus pallidus (two segments), basal nucleus of Meynert, and insular cortex
Block 4 = Amygdala (including basal nucleus of Meynert, pallidum, and hypothalamus and mammillary body)
Block 5 = Rostral midbrain (substantia nigra, cerebral peduncle and red nucleus)
Block 6 = Temporal lobe (superior and middle temporal gyri)
Block 7 = Inferior parietal lobule

DISCUSSION

In the present study, we have examined the association between Aβ deposition and ApoE genotype in 50 normal aging brains in a Malaysian population. We believe this is the first study exploring the association between ApoE ε4 and Aβ deposition in non-demented, normal aging brains in multi-ethnic Malaysia. In agreement with previous ApoE genotyping in Malaysia, the most common genotype of Malaysians is E3E3 (Table 1).[12] We found no apparent clear association between Aβ deposition and possession of the ApoE ε4 allele. Out of the four cases with Aβ deposition, only one case was heterozygous for the ε4 allele (Table 1). Conversely, there was another case with the ε4 allele but no Aβ deposition. In our study, Aβ deposition, particularly of the perivascular-type was only detected in brains aged 60 years and above. This is consistent with findings from two other studies, where the prevalence of cerebral amyloid angiopathy increased with age: 13.8% for 60-69 years, 29.2% for 70-79 years, 44.8% for over 80 years in a western population[13], and 2% for 50-59 years, 5.4% for 60-69 years, 6.7% for 70-79 years and 38.5% for over 80 years in a Malaysian population.[14] Our findings together with the predominant perivascular Aβ deposition in all the Aβ positive cases, suggest that our cases probably represent cerebral amyloid angiopathy. This is not unusual in a 'normal' elderly person as cerebral amyloid angiopathy has been reported in a large percentage in Japanese (30%)[15] and western (32.2%) populations.[13] There was also no association found between ApoE and cerebral amyloid angiopathy in a western population.[13]

The relatively low prevalence of Aβ deposition (8%) in our study is consistent with an earlier finding of Ong & Looi in which 7.9% of 114 normal aging brains above 50 years old were found to have amyloid deposition.[14] Interestingly, their sample size is more than double ours, yet the prevalence appears to be similar. In their study, both congo red staining and IHC detection techniques were used in no more than 3 blocks per case. Similar to their findings, we are unable to draw any conclusions about racial or sexual predilection for Aβ deposition although all three ethnic groups (Chinese, Indian and Malay) were involved. This relatively low prevalence of Aβ deposition appears to be consistent with the lower incidence of AD within Southeast Asia as reported in the World Alzheimer Report 2009.[8]

We are aware that our study is limited by the small number of ApoE ε4 and Aβ positive cases.

Larger sample sizes are thus required to make a more solid conclusion that there is no apparent association between ApoE ε4 genotype and Aβ deposition in the Malaysian population.

DISCLOSURE

Conflict of interest: None

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