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A prevalence study of single nucleotide polymorphisms in the promoter of the apolipoprotein E gene in different ethnic groups in Malaysia

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Abstract

Background and Objective: The promoter of the apolipoprotein E (*APOE*) gene is polymorphic at positions -491A/T, -427C/T and -219G/T. These single nucleotide polymorphisms may alter transcriptional activity and impact *APOE* expression due to differential binding of transcription factors. It has been suggested that the -491 A, -427 C and -219 T alleles are associated with a high risk of developing Alzheimer's disease. This study aims to investigate the frequencies of *APOE* promoter polymorphisms in three major ethnic groups (Malay, Chinese and Indian) in Malaysia. *Method:* DNA was extracted from blood obtained from 290 healthy people (Malay: *n*= 92; Chinese: *n*= 105; and Indian: *n*= 93), and the promoter region was amplified using PCR and genotyped by direct sequencing. *Result:* The Indian group has the lowest frequencies of - 491 A, - 427 C and - 219 T alleles (83.9%, 3.2% and 56.5%, respectively) compared to the Chinese group with the highest frequencies (97.1%, 11.9% and 67.1%, respectively). The frequencies in the Malay group were somewhere in between (94.6%, 8.2% and 61.4%, respectively). Moreover, for the - 491 and - 427 positions, the frequencies of possible genotypes viz., AA or AT or TT and CC or CT or TT, respectively, were statistically significant ($P < 0.05$, Chi- Square Test) between the 3 ethnic groups.
Conclusion: Based on the frequency of *APOE* promoter polymorphisms alone, the ethnic Indian may be predisposed to lower risks for AD than the Chinese or Malay.

INTRODUCTION

Apolipoprotein E (*ApoE*) is a polymorphic protein with three common isoforms encoded by three alleles (ε2, ε3 and ε4) of the *APOE* gene on chromosome 19q13.2.[1] *ApoE* represents a major lipoprotein within the central nervous system where it is synthesized by astrocytes.[2,3] It has been suggested that one role of *ApoE* in the brain may be neuronal homoestasis[4], particularly, mobilization of cholesterol in the central nervous system, where it is required for neuronal plasticity.[5,6] ApoE is also postulated to play a role in neuronal repair by mediating the recycle of damaged cell membranes.[4]

In-vivo and *in-vitro* studies have suggested an association of certain alleles of the *APOE* with a higher risk for Alzheimer's disease (AD).[4,7-12] The *APOE* ε4 allele is associated with both early- and late-onset AD. Factors that regulate *APOE* transcription, such as selected single nucleotide polymorphisms in the promoter or transcriptional regulatory region of *APOE*, may also contribute to an individual's risk for AD. Three single nucleotide polymorphisms in the gene promoter at positions -491 A/T, -427 C/T, and -219 G/T (also known as Th1/E47cs) have been variously reported to confer an increased risk for AD.[13-18,23,25,26] The various genotypes (allelic combinations) for each position are: -491 AA or -491 AT or -491 TT; -427 CC or -427 CT or -427 TT; -219 GG or -219 GT or -219 TT.

The allelic polymorphism at position -491 is the most thoroughly investigated. Several studies have shown that the -491 A allele is associated with an increased risk of AD that is independent of the *APOE* ε4 status.[13-17] Others have reported significant linkage disequilibrium between the -491 alleles and *APOE* ε4 polymorphisms but no independent association.[18-24] To date, the -427 allelic polymorphism has been the subject of five investigations, only two of which have shown an independent association between the -427 C allele

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and AD.[25,26] The -219 T allele is also associated with an increased risk for AD.[18,23] This allele has been proposed to act as a modifier of *APOE* ε4.[15,27] These and other findings are summarized in Table 1. Wide inter-ethnic variations in the *APOE* promoter are thought to be responsible for these inconsistent findings.[28] Overall, the relationship between *APOE* promoter polymorphisms and AD is still not fully understood.

According to the World Alzheimer Report, South and East Asian nations appear to have a lower prevalence of AD compared to more developed countries.[29] In Malaysia, there are 3 major ethnic groups: Malay, Chinese and Indian. These ethnicities, especially the latter two, have migrated to Malaysia from China and India in the last century. There are reports that ethnic differences in dementia prevalence (and presumably AD) may exist in Malaysia.[30] Differences in prevalence of various *APOE* genotypes and/or *APOE* promoter polymorphisms, among other factors, could account for some of these differences. The aim of this study is to determine *APOE* promoter polymorphisms in various races, the prevalence of which may impact on our understanding of the prevalence of AD in multi-ethnic Malaysia.

METHODS

Sample collection

A total of 290 unrelated healthy blood donors from the University of Malaya Medical Centre were recruited for this study between Jan 2009 and Dec 2010. Ethnicity was determined by careful enquiry about family genealogy over 3 generations. Two mls blood samples were collected after verbal consent from donors drawn from the three major ethnic groups in Malaysia: Malay (*n*= 92), Chinese (*n*= 105) and Indian (*n*= 93). The collected blood samples were stored

Table 1: Association of apolipoprotein E gene promoter polymorphisms with Alzheimer's disease (AD) in various countries.

COUNTRY	-491 A allele	-427 C allele	-219 T allele
Spain & USA[14]	Increased risk for AD in homozygous AA	Not studied	Not studied
Spain[33]	Independent association between the homozygous AA and AD	No association	Not studied
French[15, 27]	Increased risk for AD	No association	Increased risk for AD; Modifier of *APOE* ε4 risk
US[16]	Associated with AD (in both *APOE* ε4 carriers and non-carriers)	Not studied	Not studied
Italy[34]	Increased risk for AD	Not studied	Not studied
Australia[35]	Increased risk for AD	Not studied	No association
UK[36]	Increased risk for AD	Not studied	Not studied
Canada[22]	No association found	Not studied	Not studied
Finland[19]	No association found	Not studied	Not studied
Germany & USA[23]	No association found	Not studied	Not studied
France[26]	No association found	Independent association with AD	Not studied
China[18, 37]	No association	No association	Not studied
Japan[24]	No association		Not studied
US[17]	Independent association between the homozygous AA and AD	No association	No association
US[38]	Not studied	Not studied	No association
Spanish[25]	Not studied	Independent association with AD	Not studied

at -80°C until DNA extraction. The study protocol was approved by the Ethics Committee of the University of Malaya Medical Centre.

DNA extraction and purification

Blood samples were washed in 1X standard saline citrate buffer at room temperature and digested for 1 hour using proteinase K and sodium-dodecyl-sulphate at 55°C. Digested products were purified using phenol-chloroform-isoamyl alcohol (25:24:1, pH 8.0, Sigma, USA) extraction method.[31] The DNA was precipitated from the aqueous layer using 2M NaCI and cold absolute ethanol, air dried overnight and solubilised in 10:1 TE buffer. The purity and concentration of DNA were measured spectrophotometrically and stored at -20°C until use for PCR.

PCR and sequencing

The *APOE* promoter single nucleotide polymorphisms were determined by PCR amplification. Sense primer 5'-GGG GCT CCC CTG TGC TCA AG- 3' and anti-sense primer 5'- TGT TCT CCC CCT GCC CCA GG- 3' which flank the -491, -427 and -219 positions of the *APOE* promoter were used. The PCR was performed using Taq DNA Polymerase (Fermentas, Canada) with initial denaturation at 95°C for 3 min, followed by 35 cycles of 60 sec of denaturation at 94°C, 45 sec of annealing at 68°C, and 30 sec of extension at 72°C. After 10 min of final extension at 72°C, the PCR products were kept in 4°C. The PCR products were electrophoresed on a 1.5% agarose gel. The PCR products were then gel-purified as per manufacturer's instruction (Qiagen, USA) and sent for direct PCR sequencing (First BASE Laboratories, Malaysia). The sequence results were analysed with the Sequence Scanner (Version: 1.0, Applied Biosystems, USA).

Statistical analysis

Analysis was performed using SPSS package, version 20.0 (SPSS software, USA). Chi- square test was used to compare allele frequencies among the ethnic groups.

RESULTS

The cohort of 290 subjects in this study consisted of three major ethnic groups found in Malaysia viz., Malay (n = 92; 31.7%), Chinese (n = 105; 36.2%) and Indian (n = 93; 32.1%). The frequencies of *APOE* promoter genotypes (homozygous or heterozygous) at positions -491, -427 and -219 in the various ethnic groups are summarized in Table 2. The frequencies of each *APOE* promoter single nucleotide polymorphism in one or both alleles at positions -491, -427 and -219 are summarized in Table 3.

At the position -491, the most common and predominant genotype was homozygous AA found in 89.1% of Malay subjects, 94.3% of Chinese subjects and 69.9% of Indian subjects (Table 2). There is a statistically significant difference with regards to genotypes at position -491 between the ethnic groups ($\chi^2 = 26.0521$, $P < 0.05$). Consistent with this finding, the frequencies for A in either one or both alleles in the Malay, Chinese and Indian ethnic groups were, 94.6%, 97.1% and 83.9%, respectively. Despite having a relatively

Table 2: Frequency of *APOE* promoter genotypes in three major ethnic groups in Malaysia

Position	Genotypes	Frequency (%)			P Value (Chi- square Test)
		Malay (n= 92)	Chinese (n= 105)	Indian (n= 93)	
-491	AA	89.1	94.3	69.9	0.001 (Significant)
	AT	10.9	5.7	28.0	
	TT	0	0	2.1	
-427	TT	83.7	80.0	93.6	0.012 (Significant)
	CT	16.3	16.2	6.4	
	CC	0	3.8	0	
-219	GG	11.9	8.6	20.4	0.122 (Not Significant)
	GT	53.3	48.6	46.3	
	TT	34.8	42.8	33.3	

Table 3: *APOE* promoter polymorphism frequency at position -491, -427 and -219 and Alzheimer's disease (AD) prevalence in various populations around the world.

Ethnic Group/Country	Allele Frequency						AD Prevalence[29]
	-491		-427		-219		
	A	T	T	C	G	T	
Malay	94.6	5.4	91.8	8.2	38.6	61.4	
Chinese	97.1	2.9	88.1	11.9	32.9	67.1	
Indian	83.9	16.1	96.8	3.2	43.5	56.5	
Spain[13]	77.0	23.0	95.0	5.0	53.0	47.0	7.2 (Western Europe)
African American[39]	71.8	28.2	95.1	4.9	73.7	26.3	2.6 (Africa)
Caribbean Hispanic[39]	72.3	27.7	95.0	5.0	59.3	40.7	5.06 (Caribbean)
China[28]	87.9	12.1	-	-	-	-	3.2 (Asia, East)
France[40]	82.4	17.7	90.7	9.3	59.3	40.7	7.2 (Western Europe)
Yakut[41]	92.6	7.4	88.1	11.9	-	-	4.8 (Eastern Europe)
UK[42]	82.2	17.8	89.1	10.9	53.4	46.6	7.2 (Western Europe)
Turkey[43]	-	-	47.7	52.3	-	-	4.6 (Central Asia)
Colombia[44]	72.0	28.0	88.0	12.0	-	-	7.0 (Latin America, Southern)

higher prevalence of heterozygous AT allele (Table 2), overall, Indian subjects appear to have the lowest frequency of the -491 A allele (83.9%), an allele that has been reported to be associated with a higher AD risk.

At the position -427, the most common genotype in the three ethnic groups was homozygous TT, found in 83.7% in Malay, 80.0% in Chinese and 93.6% in Indian ethnic groups (Table 2). There is a statistically significant difference with regards to genotypes at position -427 between the ethnic groups ($\chi^2 = 12.7819$, $p < 0.05$). Consistent with this, the frequencies for C in either one or both alleles were relatively low: 8.2% in Malay, 11.9% in Chinese and 3.2% in Indian ethnic group. Thus, Indian subjects appear to have the lowest frequency of the -427 C, an allele that was reported to increase AD risk.[26] The heterozygous -427 CT was found in all races but the prevalence is relatively low, while the homozygous -427 CC was only found in Chinese subjects.

At the position -219, the most common genotype was heterozygous GT with 53.3% in Malay, 48.6% in Chinese and 46.3% in Indian ethnic groups, followed closely by the homozygous TT combination of 34.8%, 42.8% and 33.3%, respectively (Table 2). There was no statistically significant difference with regards to genotypes at position -219 between the ethnic groups ($P > 0.05$). The allele frequencies for T in either one or both alleles were 61.4% (Malay), 67.1% (Chinese) and 56.5% (Indian). Thus for the -219 T allele that is reported to be associated with a higher AD risk, its frequency appears to be lowest in the Indian group.

DISCUSSION

We studied the prevalence of *APOE* promoter

polymorphisms in 3 major ethnic groups in Malaysia and found that the Indian group appears to have relatively lower frequencies for all the alleles (-491 A, -427 C and -219 T) that have been reported to be associated with higher risks for AD. On the other hand, the Chinese group appears to have the highest frequencies of these alleles (including highest frequency for homozygous -491 AA), while the Malay group had frequencies that were somewhere in between. Based on polymorphism frequencies alone, our findings suggest that the Indian ethnic group could be predisposed to lower risks for AD than the Chinese or Malay groups, with the Chinese group having the highest risks. Interestingly, a recent prevalence study of dementia in Malaysia showed that the ethnic Indian cohort had the lowest prevalence (5.8%) compared to the Malay (14.8%) and Chinese ethnic group (6.3%).[30] In contrast, comparable dementia prevalence studies in Singapore, a neighbouring country to Malaysia, which has the same ethnic groups as well, showed that the Indian group appears to have the highest dementia prevalence (1.9%), followed by Malay (1.6%) and Chinese (1.2%) ethnic groups.[32] These apparent differences in dementia (and presumably AD) prevalence among similar ethnic groups in two neighbouring countries could be due to various factors including study design, general education levels of cohort/ethnic groups and other genetic factors such as *APOE* ε4, etc.[4,7-12] Further studies are needed to better define the relationship of *APOE* promoter polymorphisms and *APOE* 4 to AD in patients drawn from various ethnic groups. Unfortunately, to date, these studies have not been done in Malaysia.

According to the World Alzheimer Report 2009, America and European countries generally appear to have a higher prevalence of AD, whereas in China, India and Africa, the prevalence appears to be lower.[29] Generally, the frequency of the -491 A allele in Western countries is also higher than Africa. However, China also have high frequency of the -491 A allele despite the low prevalence of AD (Table 3). The frequencies of the -427 C allele and -219 T allele are also higher in America and European populations, whereas, the frequencies are lower in Africa and China. There is no information on frequencies in India. Nonetheless, these correlations, if any, are only tentative and have to be confirmed with more detailed studies.

DISCLOSURE

Conflict of interest: None

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