# Experience of ApoE Study in Thai Elderly†

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**Abstract**

The association between ApoE $E_4$ and dementia is reported in Alzheimer's disease and other dementia such as in multi-infarct dementia.

**Objectives :** To examine the association between apolipoprotein E genotype (ApoE) and dementia in Thai elderly and patients to examine the alleles frequencies of ApoE in a Thai population.

**Material and Method :** Seventy-eight cases and ninety-four controls from a community based population were recruited. Their ages were all over 50 years. Dementia was diagnosed by DSM IV criteria. Blood was taken and stored for DNA extraction and for restriction enzyme analysis of ApoE genotype. Descriptive analysis and odds ratios from SPSS 9.0 program were used in this study.

**Results :** Alleles frequencies of ApoE $E_2$, $E_3$, $E_4$ in normal controls were 0.03, 0.80, 0.17 and alleles frequencies of ApoE $E_3$, $E_4$ in dementia subjects were 0.71 and 0.29, respectively. Odds ratios for dementia risk of apolipoprotein genes were as follows: 0.62 for ApoE $E_3$ and 1.98 for ApoE $E_4$. In this study, forty-two dementia subjects had Alzheimer's disease. Fifty nine point five per cent of Alzheimer's disease subjects carried ApoE $E_4$ (positive predictive value is 0.60).

**Conclusion :** Thai elderly carry ApoE genotype distribution similar to that reported in other ethnic groups. Bearing ApoE $E_4$ gene increases the risk of developing dementia. The use of ApoE genotyping can only be a diagnostic adjunct for Alzheimer's disease.

**Key word :** Dementia, ApoE Genotype

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Apolipoprotein E (ApoE) is a polymorphic protein that plays a role in the regulation of lipid metabolism. ApoE has a special relevance to nervous tissue[1]. It is involved in the mobilisation and redistribution of cholesterol in repair, growth, and maintenance of myelin and neuronal membranes during the development or after injury[2]. ApoE is present within the plaques and dystrophic neurites in Alzheimer's disease[3]. ApoE has three major isoforms ($E_2$, $E_3$ and $E_4$) which genetically result from different alleles coding for proteins with a single amino acid substitution. Lipoprotein associated with ApoE $E_4$ cleared more efficiently than that containing ApoE $E_3$ and ApoE $E_2$. The ApoE $E_4$ has some effects on plasma cholesterol and lipoprotein levels and may alter brain reinnervation processes which rely upon cholesterol and triglyceride transported by ApoE[4]. The expression of ApoE mRNA in cultured skin fibroblasts from Alzheimer's disease and vascular dementia patients was reduced[5]. The frequency of the ApoE $E_4$ alleles is higher in Alzheimer's disease and vascular dementia patients[5]. The alleles frequency of ApoE $E_4$ is significantly higher in Alzheimer's disease patients than normal control subjects in many reported ethnic groups aged 70 or younger[6]. A higher than expected percentage of sporadic and of young onset Alzheimer's disease cases had a three-fold higher than expected likelihood of having an ApoE $E_4$ alleles[7,8]. This suggests that inheritance of ApoE $E_4$ is a risk for developing Alzheimer's disease. Another study has suggested that ApoE $E_2$ is protective or a negative risk factor[9]. However, the majority of ApoE $E_4$ individuals reach old age without impairment on the test given. In the Framingham study, the positive predictive value of ApoE $E_4$ was 0.1 for AD[10]. In the IOWA study the positive predictive value was 0.12 for cognitive impairment[11].

**Objectives**

To examine the association between apolipoprotein E genotype (ApoE) and dementia in Thai elderly and to examine alleles frequencies of ApoE in a cohort of Thai population.

## MATERIAL AND METHOD

A community based case-control study was conducted which recruited seventy-eight dementia patients and ninety-four controls. All subjects were aged over 50 years old. Dementia was diagnosed by DSM IV criteria. Dementia subtypes were diagnosed as probable Alzheimer's disease (AD),[12] vascular dementia(VAD),[13] mixed type (AD and VaD), hypothyroidism, neurosyphilis, dementia with Lewy bodies[14], Parkinson's disease dementia, and normal pressure hydrocephalus.

**ApoE genotype analysis :** Five milliliters of EDTA venous blood sample was collected and centrifuged at 3000 rpm for 10 minutes. Leukocyte DNA was extracted from packed cells according to the standard protocol. DNA was precipitated, resuspended in 10 ul of sterile distilled water, and stored at 20°C for the analysis by restriction enzyme technique. The ApoE gene segment was amplified from each sample using two oligonucleotide primers, E1 (5'GCA CGG CTG TCC AAG GAG CTG CAG GC 3', 26nts) and E2 (5' GGC GCT CGC GGA TGG CGC TGA G 3', 22 nts). Approximately 500 ng of total DNA were amplified by polymerase chain reaction (PCR) (1 min at 95°C, 1 $^1/_2$ min at 55°C and 2 $^1/_2$ min at 72°C) for 30 cycles. The amplified DNA was precipitated and the digested fragments were separated in 12 per cent polyacrylamide gel electrophoresis.

The PCR product of 271 bp in size covering nucleotide coding for amino acid codon 112 and 158 of ApoE was obtained. For ApoE $E_2$, of which amino acid codon 112 and 158 were both cysteine, the PCR product would be cleaved by restriction enzyme Hha I yielding the digested fragment of 91, 83, 30, 18, 16, 13 and 11 bp respectively. The PCR product of $E_3$, of which amino acid codon 112 and 158 were cysteine and arginine, would be digested into 91, 48, 35, 30, 18, 16, 13 and 11 bp. The digestion fragments of 72, 48, 35, 30, 19, 18, 16, 13 and 11 bp obtained from $E_4$ of which amino acid at both codon 112 and 158 were arginine.

**Data analysis :** Descriptive analysis, percentages, odds ratios and chi square tests were applied from SPSS 9.0

## RESULTS

The ages of the control group were matched with the ages of the dementia subjects. Thai mental state examination (TMSE) scores

**Table 1. Subjects' characteristics.**

| | Dementia | % | Non-dementia | % |
|---|---|---|---|---|
| Number | 78 | 45.3 | 94 | 54.7 |
| Sex : Male | 24 | 30.8 | 28 | 29.8 |
| Female | 54 | 69.2 | 66 | 70.2 |
| Age : years (mean±SD) | 69.27±8.31 | | 68.07±6.93 | |
| TMSE : mean±SD | 16.87±6.97 | | 26.92±3.40 | |

were lower in the dementia group (Table 1). The majority of the elderly had ApoE $E_3$ (3/3) genotype, sixty six per cent in the non-dementia group and 48.7 per cent in the dementia group (Table 2). ApoE (4/4) genotype was found in 5.81 per cent of the population.

Only half of these subjects had dementia. We did not find any subject who carried ApoE $E_2$ (2/2) probably due to the small sample size. Alleles frequencies of ApoE are given in Table 3. ApoE $E_4$ frequencies were higher in the dementia group than in the control group across every age group. ApoE $E_4$ allele seemed to have a bimodol distribution in the dementia group. The two peaks were found at less than 60 and 71-80 years.

Odds ratios (OR) for the risk of developing associated dementia ApoE gene are presented in Table 4. Our results showed that ApoE $E_4$ is a risk factor for dementia (OR=1.98, 95 per cent CI=1.15, 3.42). This risk is higher in the group aged 71-80 years.

In this study, 53.85 per cent of dementia subjects had Alzheimer's disease. Fifty-nine point five per cent of Alzheimer's disease patients in this study had ApoE $E_4$ either $E_3/E_4$ or $E_4/E_4$. (Positive predictive value=0.595).

## DISCUSSION

The ApoE gene is found on the long arm of chromosome 19 (19q 13.2)[16] in close relation with linkage to a late onset form of familial Alzheimer's disease (AD)[17]. ApoE was found in AD-related plaques and neurofibrillary tangles[3] and ApoE $E_4$ was reported to be associated to late onset familial AD, to sporadic AD[7], and to multi-infarct dementia[18]. A few studies have reported that the

**Table 2. ApoE genotype findings in this studied population.**

| ApoE | Dementia N | Dementia % | Non-dementia N | Non-dementia % | Total N | Total % |
|---|---|---|---|---|---|---|
| 2/2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2/3 | 0 | 0 | 5 | 5.3 | 5 | 2.9 |
| 2/4 | 0 | 0 | 1 | 1.1 | 1 | 0.6 |
| 3/3 | 38 | 48.7 | 62 | 66.0 | 100 | 58.1 |
| 3/4 | 35 | 44.9 | 21 | 22.3 | 56 | 32.6 |
| 4/4 | 5 | 6.4 | 5 | 5.3 | 10 | 5.8 |
| Total | 78 | 100 | 94 | 100 | 172 | 100 |

**Table 3. ApoE alleles frequencies (N, frequency).**

| Age | ApoE $E_2$ Dementia | ApoE $E_2$ Non dementia | ApoE $E_2$ Total | ApoE $E_3$ Dementia | ApoE $E_3$ Non dementia | ApoE $E_3$ Total | ApoE $E_4$ Dementia | ApoE $E_4$ Non dementia | ApoE $E_4$ Total |
|---|---|---|---|---|---|---|---|---|---|
| <60yrs | 0 | 0 | 0 | 10 (0.63) | 5 (0.63) | 15 (0.62) | 6 (0.37) | 3 (0.37) | 9 (0.38) |
| 60-70yrs | 0 | 4 (0.04) | 4 (0.02) | 54 (0.73) | 89 (0.78) | 143 (0.76) | 20 (0.27) | 21 (0.18) | 41 (0.22) |
| 71-80yrs | 0 | 1 (0.02) | 1 (0.01) | 36 (0.69) | 48 (0.86) | 84 (0.78) | 16 (0.31) | 7 (0.12) | 23 (0.21) |
| ≥81yrs | 0 | 1 (0.10) | 1 (0.04) | 11 (0.79) | 8 (0.80) | 19 (0.79) | 3 (0.21) | 1 (0.10) | 4 (0.17) |
| Total | 0 | 6 (0.03) | 6 (0.02) | 111 (0.71) | 150 (0.80) | 261 (0.76) | 45 (0.29) | 32 (0.17) | 77 (0.22) |

**Table 4. Odds ratios (OR) for a dementia risk of ApoE gene.**

| | | ApoE $E_2$ | ApoE $E_3$ | ApoE $E_4$ |
|---|---|---|---|---|
| OR (95%CI) | | Undefined | 0.62 (0.37, 1.06) | 1.98 (1.15, 3.42) |
| p value | | | 0.08 | 0.01* |
| Age <60yrs : | OR (95%CI) | Undefined | 1 (0.12, 7.94) | 1 (0.13, 8.08) |
| | P value | | 0.65 | 0.65 |
| Age 61-70yrs : | OR (95%CI) | 0 (0, 2.35) | 0.76 (0.36, 1.58) | 1.64 (0.77, 3.49) |
| | p value | 0.27 | 0.51 | 0.22 |
| Age 71-80yrs : | OR (95%CI) | 0 (0,18.93) | 0.38 (0.13, 1.06) | 3.11 (1.06, 9.41) |
| | p value | 0.97 | 0.06 | 0.03* |
| Age >81yrs : | OR (95%CI) | 0 (0,13.07) | 0.92 (0.08, 9.61) | 2.45 (0.17, 73.36) |
| | P value | 0.86 | 0.67 | 0.85 |

**Table 5. ApoE status in dementia group stratified by etiology of dementia (N, %).**

| | ApoE genotype | | | Total |
|---|---|---|---|---|
| | 3/3 | 3/4 | 4/4 | |
| Alzheimer's disease (AD) | 17 (40.5) | 20 (47.6) | 5 (11.9) | 42 (100) |
| Vascular dementia (VaD) | 11 (57.9) | 8 (42.1) | - | 19 (100) |
| Mixed AD&VaD | 2 (50) | 2 (50) | - | 4 (100) |
| Hypothyroidism | 1 (100) | - | - | 1 (100) |
| Neurosyphilis | 1 (50) | 1 (50) | - | 2 (100) |
| Diffused Lewy Body dementia | 1 (100) | - | - | 1 (100) |
| Parkinson's dementia | - | 2 (100) | - | 2 (100) |
| Normal pressure hydrocephalus | 3 (60) | 2 (40) | - | 5 (100) |
| Unknown | 2 (100) | - | - | 2 (100) |

increased risk for dementia associated with the $E_4$ allele is not specific to AD[10,19]. Our study demonstrated that Thai elderly have similar alleles frequencies of ApoE $E_2$, $E_3$ and $E_4$ to other ethnic groups[20]. The commonest ApoE genotype was $E_3/E_3$ in both controls and dementia subjects. In an autopsy study, the E4 alleles was carried by 75 per cent of AD patients[21]. Our study showed a 59.5 per cent prevalence rate of carrying ApoE $E_4$. It suggests that an ApoE study could be used only as an adjunct to the diagnosis of Alzheimer's disease. A previous large sample size, as well as an autopsy-proven study demonstrated that ApoE genotyping alone can not be used as a diagnostic test for AD, but when used in combination with clinical criteria, it has improved the specificity of the diagnosis[22].

Our study showed that subjects carrying ApoE $E_4$ had an increased risk of dementia not just only AD with an odd ratio of 1.98 (95% CI=1.15, 3.42). The risk was higher between 71 and 80 years old in demented persons (OR= 3.11, 9.5%CI=1.06, 9.41). The distribution of ApoE $E_4$ was bimodal in the dementia subjects. The alleles frequency of ApoE $E_4$ was higher in the demented persons who were younger than 60 years and between 71 and 80 years. This may be explained by 53.85 per cent of the dementia subjects having Alzheimer's disease and by a concept of an early and late onset form of AD in association with a genetic linkage. We could not demonstrate a protective effect of ApoE $E_2$ for dementia due to the small sample size in our study and a lower prevalence of ApoE $E_2$ than in a previous report[23].

## SUMMARY

The alleles frequencies of ApoE $E_2$, $E_3$ and $E_4$ were 0.02, 0.76 and 0.22 in Thai elderly. The ApoE $E_4$ alleles frequency was higher in dementia subjects than in normal controls (0.29 *vs* 0.17). This study confirmed a similar distribu-

tion of ApoE genotype in Thais to other ethnics. Not all Alzheimer's disease patients carry ApoE $E_4$. Hence, ApoE $E_4$ can not be used as the sole diagnostic tool for Alzheimer's disease.

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# ประสบการณ์การศึกษาสารพันธุกรรม ApoE ในผู้สูงอายุไทย†

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จากการศึกษาที่ผ่านมาพบความสัมพันธ์ระหว่าง สารพันธุกรรม ApoE E4 และภาวะสมองเสื่อมเหตุโรคอัลไซเมอร์ และยังมีความสัมพันธ์กับภาวะสมองเสื่อมจากเหตุอื่นด้วยเช่นจาก โรคหลอดเลือดสมอง

**วัตถุประสงค์** : เพื่อศึกษาความสัมพันธ์ของสารพันธุกรรม apolipoproteins E (ApoE) ต่อภาวะสมองเสื่อมในผู้สูงอายุไทย เพื่อศึกษาความชุกของสารพันธุกรรม ApoE ในประชากรไทย

**วิธีการ** : การศึกษาเป็นชนิด case-control study โดยรวบรวมผู้สูงอายุที่อายุมากกว่า 50 ปีจากชุมชนรอบบริเวณศิริราช โดยมีผู้สูงอายุ 78 ราย ในกลุ่มศึกษา และ 94 รายในกลุ่มเปรียบเทียบ กลุ่มศึกษาประกอบด้วยผู้สูงอายุที่มีภาวะสมองเสื่อม ซึ่งวินิจฉัยโรคโดยใช้เกณฑ์ชี้ตาม DSM IV และเจาะเลือดเพื่อนำมาวิเคราะห์หาสารพันธุกรรม ApoE การวิเคราะห์ข้อมูลใช้โปรแกรมสถิติ SPSS 9.0 เพื่อหาอัตราร้อยละ และค่า odds ratios

**ผลการวิจัย** : ความชุกของสารพันธุกรรม ApoE $E_2$, $E_3$, $E_4$ ในผู้สูงอายุปกติกลุ่มควบคุมเท่ากับ 0.03, 0.80, 0.17 ตามลำดับ ความชุกของสารพันธุกรรม ApoE $E_3$, $E_4$ ในผู้สูงอายุที่มีภาวะสมองเสื่อมเท่ากับ 0.71, 0.29 ตามลำดับ Odds ratios ของ ApoE $E_3$ ต่อภาวะสมองเสื่อมเท่ากับ 0.62 ของ ApoE $E_4$ เท่ากับ 1.98 ผู้สูงอายุกลุ่มศึกษา 78 ราย เป็นโรคอัลไซเมอร์ 42 ราย ซึ่งร้อยละ 59.5 ของผู้ป่วยอัลไซเมอร์นี้มีสารพันธุกรรม ApoE $E_4$ (ค่า positive predictive value เท่ากับ 0.60)

**สรุป** : การกระจายความชุกของสารพันธุกรรม ApoE ในผู้สูงอายุไทยที่ศึกษาครั้งนี้มีลักษณะเหมือนกับการกระจายที่พบในชนชาติอื่นๆที่เคยมีรายงาน การมีสารพันธุกรรม ApoE $E_4$ ทำให้เกิดอัตราเสี่ยงต่อการเกิดภาวะสมองเสื่อมสูง การตรวจ ApoE $E_4$ ในผู้ป่วยสมองเสื่อมอาจจะใช้ช่วยประกอบในการวินิจฉัยโรคอัลไซเมอร์ได้บ้าง

**คำสำคัญ** : ภาวะสมองเสื่อม, สารพันธุกรรม ApoE

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