

## Genetic Variation of Apolipoprotein E (ApoE) in Surabaya, Palu and Alor Populations of Indonesia

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### Abstract

Apolipoprotein E (ApoE) has been considered to play an important role in cardiovascular disorders. Several studies reported that genetic variation in ApoE locus influence plasma lipoprotein level. The objectives of this study was to compare the frequency of ApoE genotypes and alleles in some populations of Indonesia. One hundred and ninety five voluntarily unrelated apparently healthy individuals were recruited from Surabaya, Palu and Alor representing the western, middle and eastern populations of Indonesia, respectively. Blood samples were collected from each subject for DNA extraction. The common allelic variants of ApoE were screened using polymerase chain reaction (PCR) and restriction fragment length polymorphism. Three alleles i.e.  $\epsilon 2$ ,  $\epsilon 3$  and  $\epsilon 4$  were identified and five genotypes i.e. *ApoE*  $\epsilon 2/\epsilon 2$ , *ApoE*  $\epsilon 2/\epsilon 3$ , *ApoE*  $\epsilon 3/\epsilon 3$ , *ApoE*  $\epsilon 2/\epsilon 4$ , *ApoE*  $\epsilon 3/\epsilon 4$  were found in three populations studied, while *ApoE*  $\epsilon 4/\epsilon 4$  was absent in Surabaya, representing the western populations of Indonesia. The frequency of  $\epsilon 2$ ,  $\epsilon 3$  and  $\epsilon 4$  alleles in the western population were 0.208, 0.701 and 0.092 respectively; in the middle population were 0.242, 0.618 and 0.140 respectively and in the eastern population of Indonesia were 0.267, 0.466 and 0.267 respectively. The highest frequency of  $\epsilon 2$  and  $\epsilon 4$  allele was found in the eastern population of Indonesia. The distribution of  $\epsilon 2$  allele were not significantly different among all Indonesian populations, but significantly different were found in  $\epsilon 3$  and  $\epsilon 4$  allele in the eastern population compared to those in the western and middle populations of Indonesian. It can be concluded that the frequency of three ApoE alleles in the western and middle populations of Indonesia was not significantly different however, significantly different was observed in the frequency of ApoE  $\epsilon 3$  and  $\epsilon 4$  alleles from the eastern compared to those in the western and middle populations of Indonesia.

**Keywords :** Apolipoprotein E; genotypes; allele frequency; populations of Indonesia

### Introduction

Apolipoprotein E (ApoE), a 34-kDa glycoprotein, plays an important role in the transport and metabolism of plasma cholesterol and triglycerides through its ability to interact with cell surface of low density lipoprotein (LDL) receptor and LDL receptor-related protein (LRP). ApoE

exists in three isoforms (ApoE2, E3, and E4) that differ in their cysteine/arginine content at two polymorphic sites (Corella *et al.*, 2002). Three common *ApoE* alleles have been identified: *ApoE2*, *ApoE3*, and *ApoE4* (Elousa *et al.*, 2004). The encoded 299-residue plasma protein ApoE is a surface component of primarily triglyceride-rich lipoproteins, such as very low-density lipoproteins (VLDLs), chylomicron remnant, and high density lipoproteins (HDLs). ApoE is the main ligand for clearance of VLDLs and chylomicron remnant, and as such affects circulating concentrations of lipoproteins and

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plasma levels of cholesterol and triglycerides (Mahley & Ji, 1999). Genetic variation in the *ApoE* locus significantly affects plasma lipoprotein concentrations. The allele of ApoE is associated with increased risk of two major causes of death in low-mortality populations: ischemic heart disease and Alzheimer's disease. The presence of the  $\epsilon 4$  allele is associated with increased LDL cholesterol, whereas the presence of the  $\epsilon 2$  allele is associated with decreased LDL cholesterol and may protect against mortality due to coronary heart disease and Alzheimer's (Douglas, 2002; Jurcovicova *et al.*, 2006, Minihane *et al.*, 2007). Meta-analyses suggest that *ApoE4* carrier may have a 40-50% increased CVD risk, however the associations reported in individual studies are highly heterogeneous and environmental factors influence genotype-phenotype associations (Minihane *et al.*, 2007). Both ApoE2 and ApoE4 are also associated with increases in plasma triglycerides although the ApoE2 allele is associated with a lower CVD risk in women in the Framingham Study (Elousa *et al.*, 2004). The frequencies of these three ApoE alleles are highly variable in different populations (Singh *et al.*, 2006). Globally,  $\epsilon 3$  allele is the most frequently found, constituting 60–90% of the allelic variation,  $\epsilon 2$  allele constitutes 0–20% and  $\epsilon 4$  allele 10–20% of allelic variation (Elousa *et al.*, 2004; Gerdes *et al.*, 1996).

Indonesia is a country in Southeast Asia and Oceania, comprising 17,508 islands. Across its many islands, Indonesia consists of a variety of distinct ethnic, linguistic, and religious groups. Most Indonesians are descendent from Austronesian-speaking peoples whose languages can be traced to Proto Austronesian, which is likely originated from Taiwan. The other major grouping is Melanesians, inhabiting the eastern part of Indonesia (Dawson & Gillow, 1994). There are around 300 distinct native ethnicities in Indonesia, and 742 different languages and dialects (Merdekawaty, 2006). The objectives of this work is to study ApoE genotypes

and allele polymorphism in Surabaya, Palu and Alor populations, representing the western, middle and eastern populations of Indonesia.

### Materials and Methods

All individuals were recruited as part of a comprehensive study on the "Interaction of biochemical genetic traits and other markers in malaria endemic areas in Indonesia". All subjects were voluntarily unrelated apparently healthy individuals and all of their grandparents were born and belong to a particular ethnic in the study area. Among 195 subjects involved in the study, 82, 68 and 45 individuals were from Surabaya, Palu and Alor representing the western, middle and eastern populations of Indonesia respectively. Written informed consent was obtained from all individuals, and ethical clearance for the project was provided by Human Research and Ethics Committee of the Faculty of Medicine Universitas Gadjah Mada, Yogyakarta. Approximately 10 mL venous blood was drawn from ante cubital vein of each individuals employing EDTA anticoagulated vacutainer (Terumo). The blood was stored in 4°C and brought to the laboratory in Yogyakarta prior to laboratory examination.

### DNA analysis

Genomic DNA was isolated from blood leukocytes using a standard phenol-chloroform extraction procedure. Polymerase chain reaction (PCR) amplification was undertaken using *forward* primer: 5'-TCCAAGGAGCTGCAGGCGGCGA-3' and reverse primer: 5'-GCCCCGGCCTGTACTACTGCCA-3' to produce a 218-bp product. The mixture was a mix Promega 12.5 uL, forward and reverse primers 1  $\mu$ L, water 9.5  $\mu$ L, and DNA 1  $\mu$ L was subjected in PCR reaction. PCR conditions used in amplification were 94°C for 1 min, followed by 40 cycles of 94°C for 30 sec, 60°C for 30 sec, and 70°C for 90 sec for extension and a final extension cycle of 72°C for 10 min.

Amplified DNA 8  $\mu$ L was digested with 2.5 U Afl III and 5 U Hae II for 24 h at 37°C. The result was analyzed on 4% agarose gel and visualized by ethidium bromide staining. Digested product were 145-bp, 168-bp and 195 bp which were specific for ApoE3, E2 and E4 (Zivelin *et al.*, 1997). The genotype of ApoE in populations was compared employing Chi square analysis, and significant difference was agreed when  $p < 0,05$ .

## Results

Despite abundant reports on the genetic variation of ApoE gene locus, the distribution of ApoE alleles particularly  $\epsilon 2$ ,  $\epsilon 3$  and  $\epsilon 4$  are scarcely available in Indonesian populations, if any. In this report variation of ApoE gene locus were found in three Indonesian populations studied (Figure 1 and Figure 2). The frequency of ApoE genotypes and alleles were shown in Table 1 and Figure 2.

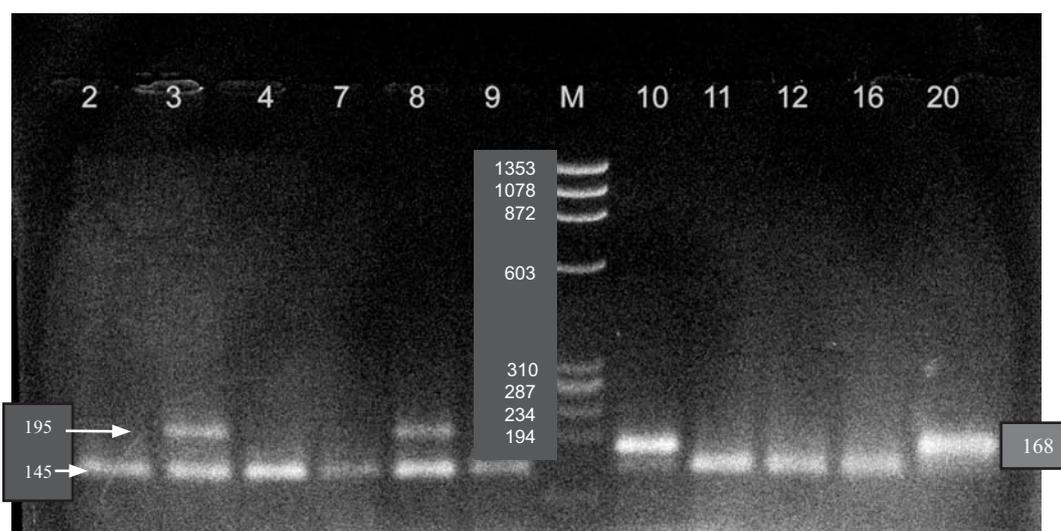


Figure 1 : Apolipoprotein E products after restriction by Afl III & Hae II enzymes.

Notes: ApoE  $\epsilon 2$  generated bands 168 and 50 bp, ApoE  $\epsilon 3$  generated bands 145, 50 and 23 bp and ApoE  $\epsilon 4$  generated bands 195 and 23 bp. No 2,4,7,9,11,12,16 were ApoE  $\epsilon 3/\epsilon 3$  genotype, no 3 and 8 were ApoE  $\epsilon 3/\epsilon 4$  genotype, no 10 was ApoE  $\epsilon 2/\epsilon 3$ , no 20 was ApoE  $\epsilon 2/\epsilon 2$  genotype and M was marker  $\phi x174$

Table 1. Distribution of apolipoprotein E (ApoE) genotypes and alleles in Surabaya, Palu and Alor representing the western, middle and eastern populations of Indonesia

Apolipoprotein E (ApoE)	Surabaya (n=82)	P Surabaya - Palu	Palu (n=68)	P Palu- Alor	Alor (n= 45)	P Surabaya- Alor
Genotype		<b>0.088</b>		<b>0.011</b>		<b>0.000</b>
$\epsilon 2/\epsilon 2$	7 (8.5%)		5 (7.4%)		7 (15.6%)	-
$\epsilon 2/\epsilon 3$	15 (18.3%)		21 (30.9%)		7 (15.6%)	
$\epsilon 3/\epsilon 3$	45 (54.9%)		25 (36.8%)		8 (17.8%)	
$\epsilon 2/\epsilon 4$	5 (6.1%)		2 (2.9%)		3 (6.7%)	
$\epsilon 3/\epsilon 4$	10 (12.2%)		13 (19.12%)		19 (42.1%)	
$\epsilon 4/\epsilon 4$	-		2 (2.9%)		1 (2.2%)	
Allele		<b>0.256</b>		<b>0.031</b>		<b>0.000</b>
$\epsilon 2$	34 (20.78%)		33 (24.2%)		24 (26.7%)	
$\epsilon 3$	115 (70.1%)		84 (61.8%)		42 (46.6%)	
$\epsilon 4$	15 (9.2%)		19 (14.0%)		24 (26.7%)	

Among total subjects examined, *ApoE*  $\epsilon 4/\epsilon 4$  genotype was not found in Surabaya population. However, despite being low frequency, it was found as low as 2.9% and 2.2% in Palu and Alor population respectively. The highest *ApoE*  $\epsilon 2/\epsilon 2$  and *ApoE*  $\epsilon 3/\epsilon 4$  genotypes frequency and the lowest *ApoE*  $\epsilon 2/\epsilon 3$  and *ApoE*  $\epsilon 3/\epsilon 3$  genotypes frequency were observed in Alor population. There were no significant difference ( $p > 0.05$ )

in *ApoE*  $\epsilon 2/\epsilon 2$  and *ApoE*  $\epsilon 2/\epsilon 3$  and *ApoE*  $\epsilon 2/\epsilon 4$  genotypes frequency between all of the populations. Significant difference ( $p < 0.05$ ) could be found in the frequency of *ApoE*  $\epsilon 3/\epsilon 3$  in all of the populations. Similarly, no significant difference of *ApoE*  $\epsilon 3/\epsilon 4$  frequency was observed in Surabaya and Palu populations. However, the distribution of *ApoE*  $\epsilon 3/\epsilon 4$  genotype frequency showed significant difference ( $p < 0.05$ ) between

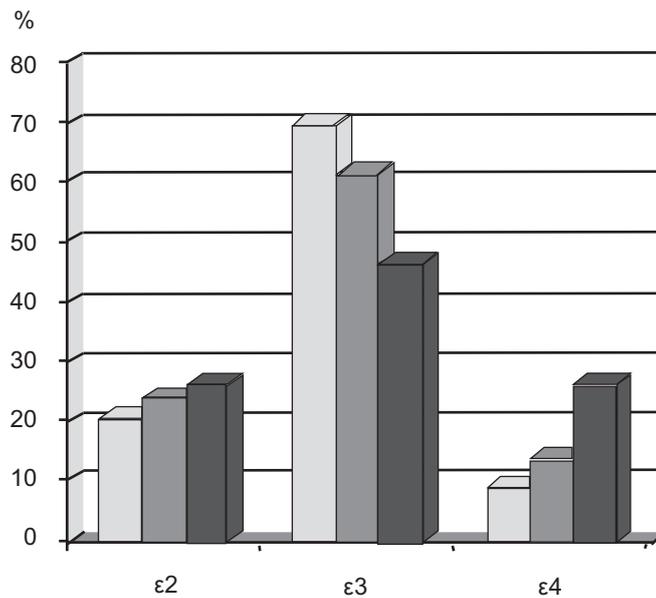


Figure 2. Distribution of ApoE alleles in Surabaya, Palu and Alor representing the western, middle and eastern populations of Indonesia (□ Surabaya ■ Palu ■ Alor)



Figure 3. Frequency of *apoE* allele in the western, middle and the eastern of Indonesia

Surabaya and Palu populations as well as between Palu and Alor population.

Having considered that the population of Surabaya, Palu and Alor represented the western, middle and eastern populations in Indonesia (figure 3), the frequency of *ApoE*  $\epsilon 2$ ,  $\epsilon 3$  and  $\epsilon 4$  alleles were not significantly different between the western and middle populations of Indonesia. Similarly, the frequency of *ApoE*  $\epsilon 2$  allele for all three populations were not significantly different ( $p > 0.05$ ). It is interesting that the frequency of *ApoE*  $\epsilon 2$  and  $\epsilon 4$  allele increased from the western to the eastern population, whereas the frequency of *ApoE*  $\epsilon 3$  allele was high in the western and declined to the eastern population of Indonesia. Significant difference in *ApoE*  $\epsilon 3$  and *ApoE*  $\epsilon 4$  alleles were found between the western and eastern populations of Indonesia as well as between the middle and the eastern populations of Indonesia ( $P < 0.05$ ). In contrast there was no significant difference for *ApoE*  $\epsilon 2$  allele frequency between the western and the middle populations of Indonesia.

### Discussion

The frequency of *ApoE*  $\epsilon 2$ ,  $\epsilon 3$ ,  $\epsilon 4$  alleles in this study were 0.207 – 0.267, 0.466 – 0.701 and 0.092 – 0.267 respectively. It can be observed from previous reports that the frequency of *ApoE* allele varied from population to population all of the world (Al-Bustan *et al.*, 2005; Belkovets *et al.*, 2001, Mastana *et al.*, 1998; Rodrigues *et al.*, 2005; Siest *et al.*, 1995, Thelma *et al.*, 2001). This present result finds high frequency of *ApoE*  $\epsilon 2$  allele in all three Indonesian populations studied compared to its frequency found in other Asians (Belkovets *et al.*, 2001). Variation of *ApoE*  $\epsilon 4$  allele frequency ranges from 5% in Taiwan & Sardinia to 40% in the Pygmies, while variation of *ApoE*  $\epsilon 3$  allele frequency ranges from 49% in Papua New Guinea to 90% in Sardinia (Siest *et al.*, 1995). In this study, the frequency of *ApoE*  $\epsilon 4$  allele in the eastern population of Indonesia was the highest compared to the other two populations. It

may be due to their relative isolation leading to endogamous mating which results in the increase frequency of rare variant. Compared with the *ApoE*  $\epsilon 4$  frequency in Papua New Guinea population (49%) it was shown that its frequency in the eastern population of Indonesia was lower. However, it was still the highest compared with the other two populations. In other word, the frequency of *ApoE*  $\epsilon 4$  allele is lower even absent in the rest of populations of Indonesia.

It has been acknowledged that populations of the western part of Indonesia were strongly influenced by Mongoloid gene pool, while those in the eastern part of Indonesia were strongly influenced by Melanesian gene pool (Sofro, 1982). If this the case, higher frequency of *ApoE*  $\epsilon 4$  allele is peculiar for Melanesian populations. The influence of these two gene pools in Indonesian populations has been also confirmed by the distribution of various genetic markers and genetic relationship analysis (Sofro, 1982).

Distribution of *ApoE*  $\epsilon 3/\epsilon 3$  genotype and the frequency of *ApoE*  $\epsilon 3$  allele in Mongolian population were reported to be higher and significantly different with Western population and Han nations in China. In addition, *ApoE*  $\epsilon 4$  allele was somewhat high in Oceania ( $0.221 \pm 0.149$ ) than in Africa ( $0.209 \pm 0.090$ ), while populations in India and other Asians showed higher *ApoE*  $\epsilon 3$  allele frequency than in European (Gerdes *et al.*, 1996).

In European, the frequency of *ApoE*  $\epsilon 4$  allele increase from the South to the North along with gradien frequency of cardiovascular disease. The *ApoE*  $\epsilon 4$  allele also involves not only in response to diet but also to other genetic factors (Couderc & Bailleul, 1998). North European (Finland, Germany) tends to have high frequency of *ApoE*  $\epsilon 4$  (14-19%) than south European (France, Italy) (7-12%). Nigeria, Japan dan Finland papulations have relative low frequency of *ApoE*  $\epsilon 2$  (3-4%). Mexican-American and American-Indian also have low *ApoE*  $\epsilon 2$  allele frequency (2-4%). In the tribes from South American-Indian, *ApoE*  $\epsilon 2$  allele

was not commonly found (Eichner *et al.*, 2002). *ApoE*  $\epsilon 2$  allele frequency in this study was 0.207 – 0.267. The study by Gajra *et al.* (1994) found  $\epsilon 2$  allele frequency in Javanese population was 0.06. *ApoE*  $\epsilon 2$  allele found high in South of Africa (0.19) (Masemola *et al.*, 2007) also in Xinjiang Uygur and Han population in China (0.193) (Yang *et al.*, 2004). The summary of the distribution of various *ApoE* allele frequency among major human population as depicted in Table 2 provides worldwide polymorphism of this gene locus.

A comprehensive population genetic study three decades ago revealed that a clinal pattern was observed in the distribution of genetic markers such as Glyoxylase (GLO), Glutamate-piruvic transaminase (GPT), Phosphogluconate dehydrogenase (PGD), and Haptoglobin (Hp) across the Indonesian archipelago. There was a trend toward increasing GPT<sup>1</sup>, PGD<sup>c</sup> and Hp<sup>1</sup> frequencies and decreasing GLO<sup>1</sup> gene frequency towards the east. For systems with more specific alleles such as Phosphoglucomutase (PGM) and Transferrin (Tf), the distribution of PGM<sup>2</sup> and Tf<sup>D1</sup> showed a western limit as far as Java and the distribution of Tf<sup>D<sup>chi</sup></sup> and HbE showed an eastern limit as far as Halmahera in the north and Timor in the south (Sofro, 1982). In a more recent study, Indonesian populations can be genetically grouped into three clusters i.e. cluster I consisted of the populations with strong influence from the Mongoloid gene pool, whose mostly living in the western

part of the country, cluster II consisted of populations who shared both Mongoloid and the Austro-Melanesian gene pool, whose living in the middle part of the country and cluster III signified by Austro-Melanesian gene pool mostly living in the eastern part of the Indonesian archipelago (Lanni 2002). *ApoE* gene polymorphism from these three respective populations also provide more or less similar pattern. No difference was observed in the distribution of *ApoE*  $\epsilon 2$  allele in all populations. The frequency of *ApoE*  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$  alleles were not different either between the western and the middle population, but the frequency of *ApoE*  $\epsilon 3$  and  $\epsilon 4$  alleles was different in the eastern populations compared to the western and middle populations.

The present study confirms genetic variability of *ApoE* genotypes and alleles across world populations. The subjects examined were all healthy individuals however, the impact of this genetic variability is important in relation to diseases. Speculation may be made and is important to clarify the association of *ApoE* gene with cardiovascular and other disorders in various populations (Abboud *et al.*, 2008; Jasinska-Myga *et al.*, 2007)

Studies investigating the relationship of *ApoE* polymorphism to vascular disease risk in different ethnic groups are also important and may provide new insights into underlying mechanisms contributing to the etiology of vascular disease (Erdembileg & Lars, 2008). This result support that the western and middle

Table 2. Distribution of various APOE alleles (frequency range) among major human populations

Population Group	$\epsilon 2$	$\epsilon 3$	$\epsilon 4$	Source
Europeans	0.044-0.119	0.64-0.898	0.052-0.310	Belkovelts <i>et al.</i> 2001
Africans	0.031-0.116	0.536-0.850	0.085-0.407	
Asians	0.02-0.140	0.620-0.870	0.070-0.240	
Native Americans	0.0-0.014	0.720-0.911	0.089-0.280	
Oceanians	0.0-0.110	0.486-0.740	0.260-0.360	
South of Africa	0.190	0.518	0.293	
<b>This research</b>				
Surabaya	0.207	0.701	0.092	
Palu	0.242	0.618	0.14	
Alor	0.267	0.466	0.267	

population of Indonesian has different gene pool with their eastern counterpart, so the characteristics of disease or management of patients might be somewhat different. This study can be extended to other populations in Indonesia with unique gene pool suffering from any disorders possibly influenced by ApoE genotype.

It is concluded that genetic variation of ApoE gene was found in three populations i.e. Surabaya, Palu and Alor, each representing populations with strong influence of Mongoloid gene pool, populations sharing Mongoloid and Austro-Melanesian gene pool and populations with strong influence of Austro-Melanesian gene pool respectively. Three alleles and five genotypes were distributed in all populations studied with the exception of *ApoE*  $\epsilon 4/\epsilon 4$  which was not found in Surabaya. The frequency of three ApoE alleles in the western and middle populations of Indonesia was not significantly different however, significant difference was observed in the frequency of ApoE  $\epsilon 3$  and  $\epsilon 4$  alleles from the eastern compared to those in the western and middle populations of Indonesia.

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