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# Apolipoprotein E as Risk Factor for Coronary Heart Disease

Pramudji Hastuti[1], Abdul Salam Mudzakir Sofro[2], Ahmad Husain Asdie[3], and Ahmad Hamim Sadewa[1]

[1]Department of Biochemistry, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2]Faculty of Medicine, Universitas YARSI, Jakarta, Indonesia
[3]Department of Internal Medicine, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

***Abstract***

Allelic variation of apolipoprotein E (apo E) has been shown to influence the concentrations of total cholesterol and low density lipoprotein cholesterol (LDL-C) and considered to play a role as one of risk factors for coronary heart disease (CHD). The aim of this study was to examine the relationship between Apo E polymorphism and the risk of CHD. Blood samples were collected from 33 CHD patients in Dr. Sardjito Hospital Yogyakarta, and 38 apparently healthy control individuals in a cross sectional study. The common allelic variants of ApoE were screened employing polymerase chain reaction and restriction fragment length polymorphism. The results obtained were analyzed by t-test and significantly different if $p < 0.05$ and risk factor was calculated by odd ratio. Frequency of *ApoE ε2, ε2* and *ε4* alleles in CHD patients were 12.1%, 69.7% and 18.2% while in controls were 18.4%, 72.4% and 9.2% respectively. Dyslipidemia condition was a strong risk factor for CHD. By controlling lipid profile and applying multifactorial statistic analysis, it was shown that *ε4* gene carrier was the risk factor for CHD, but not in triglyceride level, whereas *ε2* carrier gene was not the risk factor for CHD. Dislipidemia was the risk factor for CHD and *ApoE ε4* gene carrier was the risk factor for CHD.

**Key words**: apolipoprotein E, *ApoE ε4* gene carrier, coronary heart disease, dyslipidemia.

## Introduction

Apolipoprotein E (apoE) is a protein constituent of plasma lipoproteins that performs several functions including a role in cholesterol metabolism and as an important ligand in lipoprotein clearance. Apolipoprotein E was first identified as a constituent of very low density lipoprotein (VLDL) which function to transport triglycerides from the liver to peripheral tissues (Mayes and Botham, 2006). *Apolipoprotein E* gene is polymorphic and exists in six different isoform proteins, designated E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4 which are the gene products of three *ApoE* alleles *i.e. ε2, ε3* and *ε4* respectively (Belkovets *et al.*, 2001). Apolipoprotein E2 exhibits lower affinity for the LDL receptor, resulting in slower clearance of ApoE and higher plasma apoE levels. Conversely, ApoE4 is cleared more efficiently, resulting in lower ApoE levels and higher cholesterol levels. The genetic variations thus affect lipid metabolism and have been shown to alter risk of cardiovascular disease and dementia (Eichner *et al.*, 2002).

Epidemiologic studies addressing the contribution of *apo ε* gene to Coronary Heart Disease (CHD), reported that ~6 percent of the variation in risk for CHD in North America can be attributed to this locus. Another study of middle-aged men from nine populations estimated a ~40 percent increased risk for CHD mortality for *ε4* carriers compared with

***Corresponding author :**
Pramudji Hastuti
Department of Biochemistry, Faculty of Medicine, UGM, Sekip Utara Yogyakarta.
Phone 62-0274-6492446,
E-mail: hpramudji@yahoo.com

ε3 carriers or ε2 carriers (Stengard *et al.*, 1998). Some studies have also suggested that ε4 carriers are particularly prone to developing disseminated coronary lesions or to have an increased risk of death from CHD (Eichner *et al.*, 1993, Lehtinen *et al.*, 1995; Stengard *et al.*, 1995, Wang *et al.*, 1995). Coronary heart disease is related to dysfunction of the E4 isoform in lipoprotein metabolism and an increased concentration of serum cholesterol and triglycerides. Studies from Finland, Scotland, and northern part of Ireland have shown that populations with higher cholesterol levels and higher CHD mortality rates also have a higher frequency of the ε4 allele. Other studies have also associated the ε2 allele with increased CHD risk (Eichner *et al.*, 2002, Zannis *et al.*, 1996, Mahley *et al.*, 2006).

An association between *apoE* ε2/ε2 and type III hyperlipoproteinemia has been known for decades. This disorder is characterized by increased cholesterol and triglyceride levels, the presence of ß-VLDL (cholesterol-enriched remnants of intestinal chylomicrons and hepatic VLDL), xanthomas, and premature vascular disease, both CHD and peripheral artery disease (Mahley *et al.*, 1995). Overt hyperlipoproteinemia III occurs with a frequency of 1–5 per 5,000, whereas homozygosity for E2/2 occurs with a frequency of 0.5–1.0 per 100 in Caucasian populations. Thus, this genotype contributes to the hyperlipoproteinemia III phenotype without being its sole cause (Eichner *et al.*, 2002, Fullerton *et al.*, 2000). It examines the relationship of this genotype in CHD patients compared to the specified controls.

**Materials and Methods**

Blood samples were collected from CHD patients in Dr. Sardjito Hospital Yogyakarta compared with controls collected from an exersice group in Yogyakarta.

Inclusion criteria: patients were diagnosed by an Internal Medicine Specialist as CHD, Javanese male or female age 40 – 75 years, BMI <30, and free from Diabetes Mellitus (DM). Exclusion criteria: CHD patients with DM and non Javanese ethnic background. Inclusion criteria for the control group: apparently healthy male or female individuals, BMI < 30, no DM, age 40 – 75 years and Javanese ethnic in origin. Exclusion criteria: DM and non Javanese.

Plasma from patients and controls were separated and their lipid profiles were examined. Buffy coat from patients and controls were prepared for DNA isolation. Polymerase Chain Reaction (PCR) was performed with Zivelin *et al.*, (1997) method to amplify exon 4 and then followed by HaeII dan Afl III digestion to identify the ε2, ε3 and ε4 allele with 195 and 23 bp for ε4; 23, 50 and 145 bp for ε3 and 50 and 168 bp for ε2 respectively. Genotype of ε2/ε2 and ε2/ε3 were grouped as ε2 carrier; genotype of ε3/ε3 was grouped as ε3 carrier and genotype of ε3/ε4 and ε4/ε4 were grouped as ε4 carrier. ApoE ε2/ε4 was not included as any apoE carrier. The result was analyzed employing t-test to compare lipid profile in CHD patients and controls with significant difference at $p < 0.05$. Odd Ratio was used to determine the risk factors, while Chi Square test was used for frequency of *apoE* genotype and allele as risk factor of CHD. Another Odd Ratio was apllied to analyse *apoE* genotype and allele as risk factor of CHD with controlled lipid profile.

**Results**

Apolipoprotein E polymorphism was examined in 33 CHD patients and 38 controls. There were no significant difference between patients and controls in the body weight, height, BMI, blood pressure and blood glucose with t-test $p>0.05$ (Table 1).

There were no significant difference between CHD patients and controls in their lipid profile ($p>0.05$) despite higher levels of triglyceride, cholesterol, and LDL-C and lower level of HDL-C in CHD patients (Table 2 and Figure 1).

When Chi square analysis was employed, higher frequency of dyslipidemia in CHD patients was observed with

**Table 1**. Sex, age, body weight, height, blood pressure, and blood glucose level in CHD patients and control

| | CHD patients (n = 33) | Control (n=38) | P |
|---|---|---|---|
| Men | 20 | 22 | 0.992 |
| Women | 13 | 16 | |
| Age (year) | 60.5 ± 9.2 | 57.9 ± 8.60 | 0.843 |
| Body Weight (kg) | 63.23 ± 9.26 | 62.56 ± 9.30 | 0.754 |
| Height (m) | 1.62 ± 0.05 | 1.61± 0.053 | 0.400 |
| BMI (kg/m$^2$) | 24.01 ± 3.08 | 24.0 ± 3.35 | 0.989 |
| Systolic BP (mmHg) | 141.0 ± 17.88 | 137.31 ± 19.56 | 0.394 |
| Dyastolic BP (mmHg) | 84.0 ± 10.70 | 89.04 ± 12.96 | 0.069 |
| Blood glucose (mg/dL) | 112.4 ± 14.70 | 104.3 ± 12.5 | 0.086 |

**Table 2.** Lipid profile in CHD patients and controls

| | CHD (n = 33) | Control (n = 38) | P |
|---|---|---|---|
| Triglyceride (mg/dL) | 136.8 ± 56.6 | 123.47 ± 63.98 | 0.680 |
| Cholesterol total (mg/dL) | 196.1 ± 37.3 | 169.9 ± 39.4 | 0.657 |
| LDL-C (mg/dL) | 121.8 ± 37.3 | 102.69 ± 50.16 | 0.527 |
| HDL-C (mg/dL) | 44.8 ± 12.5 | 49.6 ± 10.1 | 0.256 |

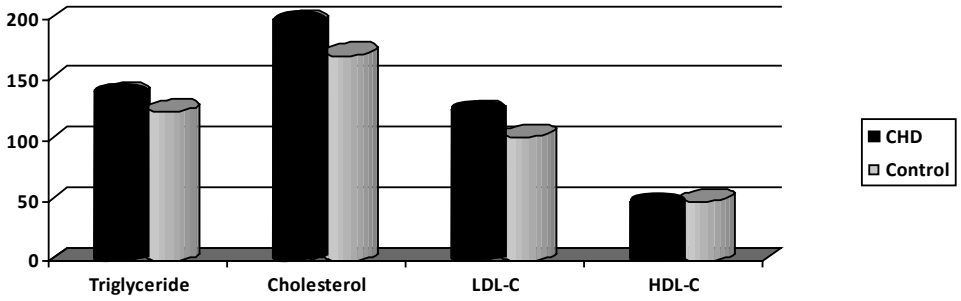


**Figure 1.** Lipid profiles in CHD and control

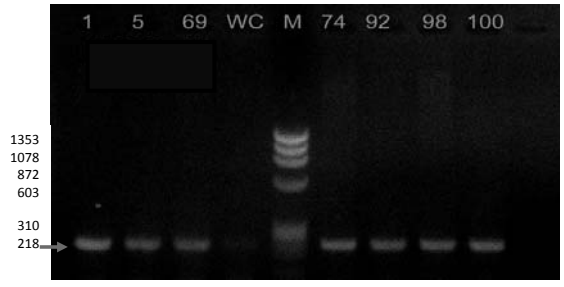


**Figure 2.** PCR result of apoE (218 base pair) (1, 5, 69, 74, 92, 98 and 100 were PCR result with 218 bp, WC was water control, M = marker Φx174)

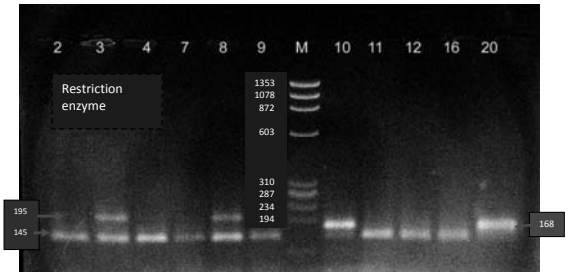


**Figure 3**. Result of PCR after digested by Afl III and Hae II enzymes (No 2, 4, 7, 9, 11, 12, 16 were *E ε3-3 genotype*, no 3 and 8 were *apoE ε3-4 genotype*, no 10 was *apoE ε2-3 genotype,* no 20 was *apoE ε2-2 geneotype*, and M was marker Φx174).

**Table 3**. Odd Ratio of lipid profile between CHD and controls

| | CHD N = 33 | CONTROL N = 38 | OR (Cl 95%) |
|---|---|---|---|
| Cholesterol >200 mg/dL | 14 | 6 | 3.93 (1.15 – 13.5) |
| < 200 mg/dL | 19 | 32 | |
| Triglyceride > 150 mg/dL | 11 | 10 | 1.40 (0.45 – 4.40) |
| < 150 mg/dL | 22 | 28 | |
| HDL-C < 40 mg/dL | 12 | 6 | 3.05 (0.88 – 10.94) |
| > 40 md/dL | 21 | 32 | |
| LDL-C > 130 mg/dL | 12 | 10 | 1.60 (0.52 – 4.98) |
| < 130 mg/dL | 21 | 28 | |

**Table 4**. Polymorphism of apoE in CHD and control

| | CHD (33) | Control (38) | OR (Cl 95%) | P |
|---|---|---|---|---|
| Genotype | ε2/ε2 : 1 (3.1%) | ε2/ε2 : 4 (10.5%) | 0.26 (0.01 – 2.68) | 0.082 |
| | ε2/ε3 : 6 (18.1%) | ε2/ε3 : 5 (13.2%) | 1.47 (0.34 – 6.36) | |
| | ε2/ε4 : - | ε2/ε4 : 1 (2.6%) | - | |
| | ε3/ε3 :14 (42.4%) | ε3/ε3 : 23 (60.6%) | 1.00 | |
| | ε3/ε4 :12 (36.4%) | ε3/ε4 : 4 (10.5%) | 4.86 (1.22 – 20.83) | |
| | ε4/ε4 : - | ε4/ε4 : 1 (2.6%) | - | |
| Gene carrier | ε2 : 7 (21.2%) | ε2 : 9 (24.3%) | 1.28 (0.40 -4.07) | 0.078 |
| | ε3 : 14 (42.4%) | ε3 : 23 (62.2%) | 1.00 | |
| | ε4 : 12 (36.4%) | ε4 : 5 (13.5%) | 3.94 (0.99 – 16.48) | |
| Alelle | ε2 : 8 (12.1%) | ε2 : 14 (18.4%) | 0.68 (0.24 -1.94) | 0.216 |
| | ε3 : 46 (69.7%) | ε3 : 55 (72.4%) | 1.00 | |
| | ε4 : 12 (18.2%) | ε4 : 7 (9.2%) | 2.05 (0.68 – 6.35) | |

significant difference ($p<0.05$) in cholesterol concentration. Applying Odd Ratio calculation, it was showed that dyslipidemia was a strong risk factor for CHD with 1.4 – 3.93 times higher (Table 3)

The result of PCR (Figure 2) was digested by Afl III and Hae II to determine the *apoE ε2*, *apoE ε3*, and *apoE ε4* genotypes (Figure 3).

The frequency of *apoE* ε2/ε2, *apoE* ε2/ε3, *apoE* ε3/ε3, and *apoE* ε3/ε4 genotypes in CHD patients were 3.1%, 18.1%, 42.4% and 36.4%, respectively and in controls were 10.5%, 13.2%, 60.6%, and 15.7%. *ApoE* ε2/ε4 and *apoE* ε4/ε4 genotypes were not found in CHD patients. Comparison was not possible. Significant difference ($p<0.05$) was only observed in *apoE* ε3/ε4 genotype with OR 4.86 times higher than other genotypes between CHD patients and controls. *Apolipoprotein E* ε2/ε2 genotype in CHD patients showed OR < 1, indicating that *apoE* ε2/ε2 genotype was protective factor for CHD (Table 4).

On the other hand, despite $p>0.05$ for *ε4* allele, OR 2.05 indicates its role as risk factor for CHD. It can be summarized that *apoE* ε3/ε4 genotype and *ε4* allele were risk factors for CHD, whereas apoE ε2/ε2 genotype and *ε2* allele were protective factors for CHD.

Being as risk factor for CHD, dyslipidemic condition especially the increase of cholesterol and LDL-C as well as the decrease of HDL-C levels were risk factors for CHD for all of apoE genotypes (Tabel 5, 6, and 7). In contrast, the increase of triglyceride level in *ε2* carrier gene was not the risk factor for CHD (Table 8).

Table 5 showed the relation of apoE polymorphism with hypercholesterolemia which is 5 and 7.88 times higher to have CHD in high concentration of cholesterol but in the case of *ε4* carrier the OR of this gene carrier could not be calculated due to low level of cholesterol in the control group. This result summarized that hypercholesterolemia caused CHD in all of *apoE gene* carrier.

**Table 5**. Risk factor of apoE polymorphism to high level of cholesterol in CHD patients and control

| ApoE gene carrier | CHD patients | Control | OR (Cl 95%) |
|---|---|---|---|
| CHOLESTEROL | | | |
| *ε2*, > 200 mg/dL | 5 | 3 | 5.00 (0.40 – 81.88) |
| *ε2*, < 200 mg/dL | 2 | 6 | |
| *ε3*, > 200 mg/dL | 6 | 2 | 7.88 (1.06 – 72.78) |
| *ε3*, < 200 mg/dL | 8 | 21 | |
| *ε4*, > 200 mg/dL | 4 | 0 | - |
| *ε4*, < 200 mg/dL | 8 | 5 | |

**Table 6**. Risk factor of ApoE polymorphism in LDL-C level in CHD patients and Control

| ApoE gene carrier | CHD patients | Control | OR (Cl95%) |
|---|---|---|---|
| LDL-C | | | |
| *ε2*, > 130 mg/dL | 5 | 3 | 5.00 (0.4 – 81.88) |
| *ε2*, < 130 mg/dL | 2 | 6 | |
| *ε3*, > 130 mg/dL | 2 | 5 | 0.60 ( 0.07 – 4.51) |
| *ε3*, < 130 mg/dL | 12 | 18 | |
| *ε4*, > 130 mg/dL | 5 | 1 | 2.86 (0.17 – 90.96) |
| *ε4*, < 130 mg/dL | 7 | 4 | |

Table 6 showed that high level of LDL-C was risk factor for CHD with *ε2* and *ε4* alleles, but not for *ε3* allele. In low HDL-C level, individual with *ε2* and *ε3 alleles* were at risk for CHD (Table 7).

**Table 7**. Risk factor for apoE polymorphism in HDL-C level in CHD patients and control

| ApoE carrier genee | CHD | Control | OR (Cl 95%) |
|---|---|---|---|
| HDL-C | | | |
| *ε2*, < 40 mg/dL | 3 | 1 | 6.00 (0.33 – 214.95) |
| *ε2*, > 40 mg/dL | 4 | 8 | |
| *ε3*, < 40 mg/dL | 6 | 3 | 5.00 (0.80 – 34.52) |
| *ε3*, > 40 mg/dL | 8 | 20 | |
| *ε4*, < 40 mg/Dl | 3 | 1 | 1.33 (0.07 – 45.29) |
| *ε4*, > 40 mg/dL | 9 | 4 | |

Table 8 showed that high level of triglyceride was the risk factor for CHD with *ε3* and *ε4* alleles, but not for *ε2* allele.

The overall OR of lipid profile showed *ε2* carrier of apoE gene was the risk factor for CHD provided that the respective individuals have high cholesterol and low HDL-C level. Similarly, carrier of *ε3* and *ε4* apoE gene were also at risk of CHD in the presense of dyslipidemia with high levels of triglycerides, total cholesterol and LDL-C and low level of HDL-C.

**Table 8**. Risk factor for ApoE polymorphism in triglyceride level in CHD patients and control

| ApoE gene carrier | CHD patients | Control | OR (Cl 95%) |
|---|---|---|---|
| TRIGLYCERIDE | | | 0.04 (0,00–0.58) |
| *ε2*, > 150 mg/dL | 1 | 8 | |
| *ε2*, < 150 mg/dL | 6 | 1 | |
| *ε3*, > 150 mg/dL | 6 | 0 | - |
| *ε3*, < 150 mg/dL | 8 | 23 | |
| *ε4*, > 150 mg/dL | 4 | 1 | 2.00 (0.11–64.91) |
| *ε4*, < 150 mg/dL | 8 | 4 | |

**Table 9**. The role of ApoE polymorphism as the risk factor for CHD with controlled lipid profile.

| Carrier of ApoE gene | OR | 95% CI |
|---|---|---|
| Gene *ε2* | 1.278 | 0.406 – 4.020 |
| Gene *ε3* | 1.000 | - |
| Gene *ε4* | 4.025 | 1.207 – 13.418 |
| **Triglyceride** | 1.016 | 1.006 – 1.027 |
| Gene *ε2* | 0.644 | 0.168 – 2.470 |
| Gene *ε3* | 1.000 | - |
| Gene *ε4* | 1.016 | 1.095 – 14,265 |
| **Cholesterol** | 1.017 | 1.003 – 1.030 |
| Gene *ε2* | 0.957 | 0.277 – 3.301 |
| Gene *ε3* | 1.000 | - |
| Gene *ε4* | 4.003 | 1.140 -14.052 |
| **HDL-C** | 0.951 | 0.908 – 0.996 |
| Gene *ε2* | 1.126 | 0.348 – 3.649 |
| Gene *ε3* | 1.000 | - |
| Gene *ε4* | 4.336 | 1.225 – 15.350 |
| **LDL-C** | 1.013 | 1.000 – 1.026 |
| Gene *ε2* | 1.117 | 0.333 – 3.743 |
| Gene *ε3* | 1.000 | - |
| Gene *ε4* | 4.119 | 1.197 – 14.165 |

This study was designed as a case control study without special treatment to control lipid profile in both groups which was later controlled by multifactorial analysis. Table 9 showed even in controlled lipid profile, ε*4* gene carrier has the risk factor for CHD 3.94 times higher except in triglyceride level, while the ε*2* gene carrier was not the risk factor for CHD with OR <1.

**Discussion**

To demonstrate the role of *ApoE* polymorphism as the risk factor for CHD, the frequency of *ApoE* gene in CHD patients was compared with controls. It was shown that ε*2* allele was the protective factor for CHD and ε*4* allele was the risk factor for CHD. This risk factor was not different in other world populations, in which ε*4* gene was the risk factor for CHD than those ε*2* and ε*3* genes (Eichner *et al*., 2002; Elousa *et al*., 2004; Mahley *et al*., 2006; Mc. Neale *et al*., 2000; Pirim *et al*., 2001). This ε*4* gene was also the risk factor for myocard infarc, atherosclerosis, stroke, and neurodegeneration (Elousa *et al*., 2004; Frikke-Schmidt *et al*., 2000 (a); Guera *et al*., 2003; Leshinsky-Silver *et al*., 2006; Mahley *et al*., 2006; Masemola *et al*., 2007; Moghadasian *et al*., 2001; Sheehan *et al*., 2000; Sima *et al*., 2006; Yang *et al*., 2004). Population with high frequency of ε*4* allele have high incidence of ischemic heart disease and this genetic determinant was related to mortality in isolated populations (Garces *et al*., 2004). Different result were found in China, where polymorphism of ε*4* gene was not the risk factor for CHD (Liu *et al*., 2003), as well as in coronary artery disease. This ε*4* gene was not the risk factor in Oman, Greek and Brazalian populations (Al-Yahyaee *et al*., 2007; De Franca *et al*., 2004; Kolovou *et al*., 2002; Souza *et al*., 2007)

The role of *apoE* polymorphism in causing dyslipidemia was studied in CHD patients and controls. A*polipoprotein ε2* allele has protective effect for CHD, but ε*3* and ε*4* alleles were the risk factor for CHD especially in individuals with dyslipidemia. The role of apoE polymorphism on dyslipidemia in Indonesian populations seems to be almost similar to that in the world's populations in which the ε*2* allele was the protective factor for CHD despite suffering from dyslipidemia (Chaaba *et al*., 2009; Chanprasetyothin *et al*., 2000; Eichner *et al*., 2002; Elousa *et al*., 2004; Mahley *et al*., 2006; Masemola *et al*., 2007; Moghadasian *et al*., 2001; Rodsari *et al*., 2005; Sheehan *et al*., 2000; Yang *et al*., 2004; Zannis *et al*., 1996). Other studies reported that ε*2* allele was related with high triglyceride level and the incidence of type III hyperlipoproteinemia (Batal *et al*., 2000; Bennet *et al*., 2007; Eichner *et al*., 2002; Letonja *et al*., 2004; Liberopoulos *et al*., 2004; Pallaud *et al*., 2001).

The role of apoE polymorphism in causing dyslipidemia is due to the ability of apoE3 to accept more cholesterol from fibroblast than apoE2 and apoE4 (Huang *et al*., 2009). In $HDL_3$, apoE3 binds cholesterol better than apoE4, because the structure of carbon end domain of apoE4 was irregular and more exposed to the water; these differences causes the pathologically of cardiovascular and neurodegenerative disease (Sakamoto *et al*., 2008). These conditions showed that genetic factors and lipid profile varies with age, sex, and the differences of environmental factors (Pallaud *et al*., 2001). Studies by Eichner *et al*., (2002), giving hypolipidemic drugs to block HMG-CoA reductase or drugs to reduce lipid profiles, was effective for *apoE* ε*2* and *apoE* ε*3* gene carriers, but *apoE* ε*4* gene carrier was difficult to be influenced by medical intervention. Low lipid and cholesterol diets induced the decrease of cholesterol and LDL levels higher in *apoE* ε*4* gene carrier than in *apoE* ε*2* and *apoE* ε*3* gene carriers. It was shown that response to hypolipidemic drugs was different in apoE polymorphism. Treatment with phenofibrate showed that ε*2* allele reduce lower in triglyceride level than others (Irvin *et al*., 2010). The defect in E4 protein, causes efficacy to bind and transport of lipid decrease. Statin drug to decrease lipid profiles was not responded by some individuals because of high response variability of hypolipidemic drugs. It can

be summarized that the detection of genetic variability that influence lipoprotein levels in the plasma supports to predict therapeutic response (Morrison, 2007). The ε*4* allele is consistently lower in reduced cholesterol level after hypolipidemic therapeutic in Portugese (Withers, 2011). Regarding the effect of therapeutic intervention to modify the disease related with apoE polymorphism, *apoE* ε*4* gene carrier had the worst effect (Cacabelos *et al*., 2010)

As conlusion, dyslipidemia was the risk factor for CHD. *Apolipoprotein E* ε*3/*ε*4* genotype and ε*4* allele were the risk factor for CHD whereas *apoE* ε*2/*ε*2* genotype and ε*2* allele were protective factor for CHD. Polymorphism of *apoE* led to variability of triglyceride level but not causes variability of other lipid profile. *Apolipoprotein E* ε*3/*ε*4* genotype and ε*4* allele were the risk factor for CHD.

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