International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:10, No:9, 2016

Apolipoprotein E Gene Polymorphism and Its Association with Cardiovascular Heart Disease Risk Factors in Type 2 Diabetes Mellitus

Amani Ashari, Julia Omar, Arif Hashim, Shahrul Hamid

***Abstract*—**Apolipoprotein E (APOE) gene polymorphism has influence on serum lipids which relates to cardiovascular risk. The purpose of this study was to determine the frequency distribution of APOE alleles among Malaysian Type 2 Diabetes Mellitus (DM) patients with and without coronary artery disease (CAD) and their association with serum lipid profiles. A total of 115 patients were recruited in which 78 patients had Type 2 DM without CAD and 37 patients had Type 2 DM with CAD. The APOE polymorphism was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The APOE ε3 allele was the most common one in both groups. There was no significant association between the APOE genotypes and the CAD status in Type 2 DM using Pearson χ^2 test. Further analysis indicated there were no significant differences in all lipid parameters between E2, E3 and E4 subgroups in both groups. The study showed that the E4 allele carriers of Type 2 DM with CAD patients had higher LDL-C level and lower HDL-C level compared to the other allele carriers. However, analyses showed these levels were not statistically different. The study also showed that the Type 2 DM with CAD group with E2 allele had higher triglyceride (TG). In conclusion, further study with larger sample size is needed to confirm role of E4 as a marker of CAD among Type 2 DM patients in Malaysian population.

***Keywords*—**Apolipoprotein E, diabetes mellitus, cardiovascular disease, lipids.

I. Introduction

PREVALENCE of diabetes has increased from 5.1% in 2003 to 6.0% in 2007 and it is estimated to increase to 7.3% by 2025 [1]. The International Diabetic Foundation (IDF) predicts Malaysia among the top 10 countries with highest prevalence of diabetes between age of 20-79 years old by 2030 [2]. In addition, the World Health Organisation (WHO) has estimated that in 2030, Malaysia would have a total of 2.48 million people with diabetes [3].

In Malaysia, the Third National Health and Morbidity Survey (NHMS III) showed that the prevalence of Type 2 DM among adults aged 30 years and above was 14.9% in 2006, compared to 8.3% in 1996. It has increased by 80% over a decade, representing an average 8% rise per year [1]. There are two aspects to the clinical manifestations of DM which relate to the metabolic disturbance (e.g. hyperglycemia, glycosuria which resulting in osmotic diuresis that causes the classic symptoms of polyuria and polydipsia) and those related to the long term complications. Long-term complications of DM can be divided into two which are microvascular complications (i.e. nephropathy, neuropathy and retinopathy) and macrovascular disease related to atherosclerosis (i.e. cardiovascular, cerebrovascular, peripheral vascular disease). Risk of macrovascular disease is greater in Type 2 DM than Type 1 DM [1]. An important factor for an increased cardiovascular disease (CVD) risk associated with diabetes is diabetic dyslipidemia. A common cluster of harmful changes in lipid metabolism is frequently noted in patients with diabetes. Plasma lipids alteration in patients with Type 2 DM were found to be associated with CAD, cerebrovascular disease and nephropathy [1]. Every 1 mmol/L reduction of low-density lipoprotein-cholesterol (LDL-C) decreased 36% risk of developing CAD. It was reported that 1 mmol/L increase of fasting TG increased 76% CAD risk for women and 32% CAD risk for men [1].

Poorly managed Type 2 DM leads to modifications of lipoprotein metabolism. APOE polymorphism has been widely studied genetic risk factor in humans due to its well-established links to Alzheimer's dementia, dyslipidemia, and CAD [1]. APOE polymorphism is proven to correlate with lipid metabolism disturbances and with coronary artery stenosis. Furthermore, investigators found that ε4 allele carriers had reduced survival rates after myocardial infarction (MI). Notably, the derived benefit from statin therapy did not relate to greater lipid lowering, which provides further evidence for a pleiotropic effect of statin use [1]. Preliminary data suggest that ε4 allele carrier identification could be beneficial for CAD risk prediction and institution of statin therapy in patients with moderate risk factors for IHD. Numerous studies have been done; however, results varied between studies as well as in different populations. In Malaysia, study on APOE genotyping is still limited especially in Type 2 DM patients with CAD. Therefore, the aim of this study is to determine distribution of APOE polymorphism in Type 2 DM and particularly the influence of APOE genotypes on the lipid profiles in a group of Malay Type 2 DM patients and risk of developing CAD.

II. Materials and Methods

A. Study Design

A case control study was done to determine the frequency distribution of APOE genotypes and alleles in patients with Type 2 DM with and without CAD. Restriction fragment length polymorphism (RFLP) of APOE gene and biochemical

Shahrul Hamid is with the Advanced Medical and Dental Institute, Universiti Sains Malaysia, Malaysia (e-mail: shahrulbariyah@usm.my).

analyses were done to determine the association of APOE genotypes and alleles with serum lipid levels. A total of 116 patients comprising of 78 Type 2 DM without CAD and 38 Type 2 DM with CAD were recruited at Hospital Kuala Lumpur.

B. Patient Selection Criteria

Patients diagnosed with Type 2 DM without CAD and Type 2 DM with CAD aged between 25 to 60 years old. Diagnosis of CAD was made according to the Malaysian Ministry of Health guideline. Diagnosis of heart disease was based on fulfilment of at least 2 out of 3 criteria which are typical clinical history, ECG changes and raised cardiac biomarkers. Patients are excluded from the study if they meet any of the following conditions: Smoker, alcohol consumer, diagnosed with secondary hyperlipidemia (chronic kidney disease stage 4, nephrotic syndrome, hypothyroidism), patients with suspected primary hyperlipidemia clinically (obvious tendon xanthomata, tuberous xanthomata) and pregnant or those on contraceptive drugs.

C. Biochemical Analyses

Samples collected in EDTA tube were kept at 4 °C and sent to HKL Chemical Pathology Laboratory. DNA was extracted from blood within 1 week. APOE genotyping was done based on method described by Hixson and Vernier with some modification [1]. Total cholesterol (TC), TG and high density lipoprotein cholesterol (HDL-C) were assayed by an enzymatic method on the same day of sample collection using Cobas 8000 Modular Analyzer Series at HKL Core Laboratory. The LDL-C was calculated by using the Friedewald's equation. For each test, quality control was performed for assay validation.

D.APOE Genotyping

DNA was extracted from whole blood using QIAGEN Protease after preparation of buffy coat layer (leucocyte-enriched fraction of whole blood) by centrifugation. Five hundred microlitre of whole blood was pipetted into microcentrifuge tube then centrifuged at 8000 rotors per minute (rpm) for 2 minutes to obtain three different fractions composed of plasma, buffy coat and concentrated erythrocytes. Three hundred microlitre of plasma and red blood cells were discarded and 20 µl of protease and 200 µl buffer were added into the remaining 200 µl sample. It was mixed immediately and incubated at 56 ºC for 10 minutes. Next, tube was centrifuged and 200 µl of absolute ethanol (96-100%) was added and mixed immediately by using vortex for 15 seconds. It was then centrifuged again. The tube was then transferred to the QIAamp Spin Column into the 2 ml collection tube and centrifuged at 8000 rpm for 1 minute. Later the column was transferred into a new 2 ml collection tube and 500 µl buffer AW1 was added. A lid was placed and centrifuged at 8000 rpm for 1 minute. This was followed by addition of 500 µl Buffer AW2 and tube was centrifuged again at 14000 rpm for 4 minute. Column was placed into the 1.5 ml microcentrifuge tube and the collection tube that contained filtrate was removed. The column was added with 80 µl of Buffer AE and left at room temperature for 1 to 5 minutes and subsequently centrifuged at 8000 rpm for 1 minute. DNA purity was determined by using Nanodrop ND 1000 Spectrophotometer. DNA sample was then kept at -20 ºC until use for DNA amplification. The APOE gene sequence was amplified by using PCR. The oligonucleotide primers used for amplification were - Primer F4; 5'-ACA GAA TTC GCC CCG GCC TGG TAC AC-3' (forward primer) and Primer F6; 5'-TAA GCT TGG CAC GGC TGT CCA AGG A-3' (reverse primer) at concentration of 1.0 µl (2.0 µM) each. Five microlitre of DNA was used as template DNA and added to PCR mixture consisting of 28.7 µl double distilled (Mili-Q) water, 1.5 µl (2.5 mM) magnesium chloride (MgCl), 5 µl of 10X PCR buffer (without MgCl), 5 µl dimethyl sulfoxide (DMSO), 2.0 µl (0.4 mM each dNTP) dinucleotide triphosphates (dNTPs), oligonucleotide primers and 0.8 µl (4U) Taq DNA Polymerase. Each reaction mixture was heated at 95 ºC for 5 minutes for denaturation, followed by 30 cycles of amplification by primer annealing at 60 ºC for 1 minute, extension at 70 ºC for 2 minutes and denaturation at 95 ºC for 1 minute. The restriction enzyme HhaI digested products (10 µl) were mixed with 1.0 µl of loading dye and electrophoresed on 3% agarose gel at 90V for 60 minutes. DNA band image was captured by using Image Analyzer System, Fluorchem 5500. Finally, APOE genotype was determined based on its fragment sizes.

E. Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 22.0 software package (SPSS Inc., Chicago, IL). The *p* value less than 0.05 was considered as statistically significant at 95% confidence interval. All the numerical data were presented as median (IQR), while categorical data expressed as number (%). The nonparametric Mann-Whitney test was used for difference in lipid profiles in Type 2 DM without CAD and Type 2 DM with CAD group. Distribution of APOE genotypes in Type 2 DM patients without CAD and Type 2 DM with CAD were analyzed by Pearson Chi-square ($\chi 2$) test. Association was compared between three alleles using Kruskal-Wallis analysis in both groups.

III. Results

A. General Characteristics of Study Patients

A total of 78 Type 2 DM without CAD patients and 37 Type 2 DM with CAD that met the inclusion criteria and gave written consent were enrolled in the study. Male constituted the majority of the study population accounting for 62.6% (n= 115) of the study population. Our study population comprised of 59.1% Malay, 27% Indians and 13.5% Chinese. It was noted among Type 2 DM without CAD group the Malay ethic group was 60.3% followed by 25.6% Indian and 14.1% Chinese. Similar pattern of distribution was noted among the Type 2 DM with CAD group with highest percentage in Malay with 56.8% followed by 29.7% Indian and 13.5% Chinese. The median age for Type 2 DM without CAD group

was 50 years old while 54 years old for Type 2 DM with CAD group. The median duration for treatment of each group were the same. Median fasting blood glucose and HbA1c were higher in Type 2 DM without CAD group. The general characteristics of recruited patients are shown in Table I.

TABLE I
GENERAL CHARACTERISTICS OF THE OVERALL STUDY PATIENTS

Variables	T2DM		Total n (%)
	CAD n (%)	No CAD n (%)	
Age (years)	54.0 (12.0) [a]	50.0 (12.0) [a]	115 (100.0)
Height (cm)	168.0 (18.0) [a]	165.0 (13.0) [a]	115 (100.0)
Weight (kg)	78.0 (10.0) [a]	73.0 (15.0) [a]	115 (100.0)
Gender			
Male	25 (67.6)	47 (60.3)	72 (62.6)
Female	12 (32.4)	31 (39.7)	43 (37.4)
Ethnicity			
Malay	21 (56.8)	47 (60.3)	68 (59.1)
Chinese	5 (13.5)	11 (14.1)	16 (13.9)
Indian	11 (29.7)	20 (25.6)	31 (27.0)
Duration (year)	7.0 (6.5) [a]	7.0 (9.0) [a]	115 (100.0)
FBS (mmol/L)	7.1 (5.4) [a]	8.5 (5.0) [a]	115 (100.0)
HbA1c (mmol/L)	7.4 (1.9) [a]	8.1 (3.3) [a]	115 (100.0)

Values are expressed as number (%) or a median (interquartile range). FBS = fasting blood sugar; HbA1c = glycosylated hemoglobin; T2DM = Type 2 DM.

B. Lipid Profile

In this study, TC, TG and LDL-C levels were significantly different between Type 2 DM without CAD and Type 2 DM with CAD group, calculated using Mann-Whitney Test, where $z = -2.61$ and $p = 0.009$; $z = -3.02$ and $p = 0.003$; and $z = -2.32$ and $p = 0.009$ respectively. The median for TC, TG and LDL-C were higher in Type 2 DM without CAD group. There was no significant difference in HDL-C level between Type 2 DM without CAD and Type 2 DM with CAD group, assessed by Mann-Whitney Test, whereby $z = -1.83$ with $p = 0.067$. The lipid profiles of the study patients are as summarized in Table II.

TABLE II
LIPID PROFILES IN TYPE 2 DM WITH CAD AND WITHOUT CAD

Lipid parameters (mmol/L)	T2DM		Z statistic[a]	*p*-value
	CAD Median (IQR)	No CAD Median (IQR)		
TC	4.30 (1.90)	5.05 (1.53)	-2.61	0.009
TG	1.40 (0.70)	1.85 (1.12)	-3.02	0.003
HDL-C	1.00 (0.40)	1.10 (0.30)	-1.83	0.067
LDL-C	2.50 (1.75)	3.10 (1.30)	-2.32	0.020

[A] Mann-Whitney test was applied, statistical significant at p value < 0.05. Values are expressed as median (inter quartile range). T2DM = Type 2 diabetes mellitus.

C. Frequency Distribution of APOE Genotypes in Type 2 DM Patients with and without CAD

From this study, it was observed that the commonest APOE genotype in both groups were E3/E3 (59.1%), followed by E3/E4 (21.7%), E2/E3 (11.3%), E2/E2 (4.3%) and both E4/E4 and E2/E4 (1.7%). The E2/E4 was detected only in Type 2 DM without CAD group. APOE genotypes distribution in Type 2 DM without CAD group was E3/E3 (59%), E3/E4 (20.5%), E2/E3 (12.8%), E2/E2 (3.8%), E2/E4 (2.6%) and E4/E4 (1.3%). APOE genotypes distribution in Type 2 DM with CAD group was similar to non CAD group with E3/E3 (59.5%), E3/E4 (24.3%), E2/E3 (8.1%), E2/E2 (5.4%) and E4/E4 (2.7%). Data for APOE genotypes frequency distribution in Type 2 DM with and without CAD were shown in Table III.

TABLE III
DISTRIBUTION OF APOE GENOTYPES IN TYPE 2 DM PATIENTS WITH CAD AND WITHOUT CAD

APOE Genotypes	T2DM		Total
	CAD n (%)	No CAD n (%)	
E2/E2	2 (5.4)	3 (3.8)	5 (4.3)
E2/E3	3 (8.1)	10 (12.8)	13 (11.3)
E2/E4	0 (0.0)	2 (2.6)	2 (1.7)
E3/E3	22 (59.5)	46 (59.0)	68 (59.1)
E3/E4	9 (24.3)	16 (20.5)	25 (21.7)
E4/E4	1 (2.7)	1 (1.3)	2 (1.7)
Total	37 (100.0)	78 (100.0)	115 (100.0)

Values are expressed as number (%). T2DM = Type 2 DM.

The distribution of E2, E3 and E4 subgroup were compared by Pearson Chi-square ($\chi 2$) test. For comparison of E2 with E3 subgroup, the p value was 0.710; for comparison of E2 with E4 subgroup, the p value was 0.519 and for comparison of E3 with E4 subgroup, the p value was 0.663. There were no significant association observed in this study between APOE genotypes and CAD status in Type 2 DM using Pearson $\chi 2$ test.

D. Association between Different APOE Genotypes with Serum Lipid Profiles in Type 2 DM Patients

TABLE IV
ASSOCIATION BETWEEN DIFFERENT APOE GENOTYPES WITH LIPID PARAMETERS IN TYPE 2 DM WITH CAD GROUP

Lipid parameters	APOE subgroup	N	Median (IQR)	Chi-Square (X^2) (df)[a]	*p*-value
TC (mmol/L)	E2	5	4.70 (2.60)		
	E3	22	3.95 (1.98)	1.69 (2)	0.429
	E4	10	4.65 (2.00)		
TG (mmol/L)	E2	5	3.60 (2.20)		
	E3	22	1.35 (0.80)	4.52 (2)	0.104
	E4	10	1.30 (0.63)		
HDL-C (mmol/L)	E2	5	0.90 (0.25)		
	E3	22	1.00 (0.32)	3.44 (2)	0.545
	E4	10	0.85 (0.75)		
LDL-C (mmol/L)	E2	5	2.50 (2.05)		
	E3	22	2.30 (1.33)	1.21 (2)	0.180
	E4	10	2.95 (2.45)		

[a] Kruskal-Wallis test was applied. E2 = Genotypes E2/E2 and E2/E3; E3 = Genotypes E3/E3; E4 = Genotypes E4/E4 and E3/E4; E2/E4 (n=2) were not included.

Association was compared between the three alleles using Kruskal-Wallis analysis in both Type 2 DM with and without CAD group. In the Type 2 DM with CAD group, there were no significant difference in all lipid parameters between E2, E3 and E4 subgroups. However, E2 subgroup showed higher in TG level and E4 subgroup showed higher in LDL-C level,

but it did not reach statistical significance as shown in Table IV. Similarly, in the Type 2 DM without CAD, there were also no significant differences in TC, TG, HDL-C and LDL-C between E2, E3 and E4 subgroups. Although it was not statistically insignificant difference, the E3 subgroup tend to show higher level of LDL-C as shown in Table V.

TABLE V
ASSOCIATION BETWEEN DIFFERENT APOE GENOTYPES WITH LIPID PARAMETERS IN TYPE 2 DM WITHOUT CAD GROUP

Lipid parameters	APOE subgroup	n	Median (IQR)	Chi-square (X^2) (df)[a]	*p*-value
TC (mmol/L)	E2	13	4.80 (1.55)	3.00 (2)	0.223
	E3	46	5.30 (1.53)		
	E4	17	4.40 (1.40)		
TG (mmol/L)	E2	13	2.00 (1.05)	1.24 (2)	0.539
	E3	46	1.70 (1.23)		
	E4	17	2.20 (1.00)		
HDL-C (mmol/L)	E2	13	1.10 (0.35)	0.71 (2)	0.703
	E3	46	1.10 (0.30)		
	E4	17	1.00 (0.30)		
LDL-C (mmol/L)	E2	13	2.80 (1.20)	5.65 (2)	0.059
	E3	46	3.40 (1.15)		
	E4	17	2.70 (1.25)		

[a] Kruskal-Wallis test was applied. E2 = Genotypes E2/E2 and E2/E3; E3 = Genotypes E3/E3; E4 = Genotypes E4/E4 and E3/E4; E2/E4 (n=2) were not included TC.

IV. DISCUSSION

First part of the study was to determine lipaemic and glycaemic levels in Type 2 DM patients without CAD and Type 2 DM with CAD Percentage of male was higher than female patients in both groups. Malay accounts for the majority of the study subjects in both groups followed by Indian and Chinese. This differs from the Malaysian National Health Morbidity Survey III (NHMS III) report in 2006, whereby the Indian ethnicity showed the highest prevalence followed by Malays and Chinese [2]. The median duration of diabetic for both groups was almost the same. Age and sex are considered as risk factors for CAD because the rates are higher in the older age group and higher in men than women of the same age [9]. In our study, the median age for Type 2 DM with CAD was slightly higher compared to the other group. The study also showed in Type 2 DM with CAD group, men comprised about 67.6% of the total. However, it was noted that diabetic control was better among Type 2 DM with CAD group compared to those having Type 2 DM without CAD. This was reflected by median of TC, TG and LDL-C, which were lower in Type 2 DM with CAD group. For patients with diabetic complication, the physician need to aim for good control of glycemic and lipid profiles. This could possibly be the reason for Type 2 DM with CAD group to have better diabetic control in terms of glycemic level and lipid profiles. Incidence of clinical complications Type 2 DM was significantly associated with glycaemic control. In patients with Type 2 DM, previous prospective studies have shown an association between the degree of hyperglycaemia and increased risk of microvascular complications [10], CAD [11] and macrovascular mortality [12]. Each 1% reduction in HbA1c was associated with reduction in risk by 21% for death related to diabetes, 14% for MI and 37% for microvascular complications [13]. The ADA guidelines recommends HDL-C level of 1.15 mmol/L in men and 1.4 mmol/L in women, LDL-C level of < 2.6 mmol/L and TG level of <1.71 mmol/L as clinical targets for lipids in Type 2 DM [14]. In this study, the median for Type 2 DM without CAD group was outside of clinical target. Median for TG and LDL-C were lower in Type 2 DM with CAD and statistically different with the other group. This was due to Type 2 DM group were on aggressive treatment for reduction of LDL-C as recommended by NCEP ATP III [9]. However, we noted in both groups, HDL-C level was still below target level. The Type 2 DM with CAD group had lower HDL-C than Type 2 DM without CAD, although the difference was not statistically significant. Data from the lipid profiles of Type 2 DM without CAD showed pattern of lipid abnormality was high for LDL-C, low for HDL-C and high for TG compared to Type 2 DM with CAD group. Previous study at Hospital Universiti Sains Malaysia (HUSM) among diabetic patients found that the most common pattern of lipid values was an isolated increase in LDL-C. The second most common pattern was a combination of high LDL-C and low HDL-C. Followed by combination of high LDL-C, low HDL-C and high TG [15]. Cook et al. reported most common lipid abnormality was high LDL-C with low HDL-C. Second was isolated increase in LDL-C, followed by patients had high LDL-C, low HDL-C and high TG [16].

Second objective of this study was to compare the frequency distribution of APOE alleles between Type 2 DM patients without CAD and Type 2 DM with CAD. APOE genotypes were grouped into 3 subgroups for comparison of APOE allele frequency distribution which were E2 group consisted of E2/E3 and E2/E2 patients, E3 group consisted of E3/E3 patients, and E4 group consisted of E3/E4 and E4/E4 patients. E2/E4 genotype was excluded from analysis as suggested by previous report as they could not be simply classified into any single allele group [17]. The other factor was due to the opposite effect of each alleles as mentioned in previous studies [18]. In this study, the rare genotype E2/E4 as reported in literature was observed only among Type 2 DM without CAD group [19], [20]. Frequency of APOE genotypes in the overall study population showed that the most frequent APOE genotypes was E3/E3 (59.1%). Findings from this study were in consistent with previous studies [21]-[23]. The next most common APOE genotype observed was E3/E4 (21.7%), followed by E2/E3 (11.3%), E2/E2 (4.3%) and both E2/E4 and E2/44 (1.7%). Generally, there was no significant differences in APOE frequency distribution between Type 2 DM without CAD and Type 2 DM with CAD group. However, percentage of those with ε3 carrier (E2/E3, E3/E3 and E3/E4) was higher in Type 2 DM without CAD group. A few studies indicate higher frequency of APOE-ε4 in CAD/Type 2 DM and CAD/non-diabetic patients, whereas others fail to detect any difference [24]. APOE gene polymorphism that confers susceptibility to or protection from

CAD in patients with Type 2 DM and non-diabetic might differ with ethnicity [25]. Seet et al. showed that Indian subjects had a higher frequency of the ε4 allele (14.3%) compared to other ethnic groups with 13.54% in the Malays and 9.6% in the Chinese. They also appeared to have higher frequency of the ε3 allele (85.7%), whereas the Chinese had higher frequency of ε2 allele (7.0%) [23]. Previous study reported that there was no statistically significant relationship between APOE polymorphism with MI and ε2 allele but the E2/E3 genotype showed trend towards lower risks of MI compared to the E3/E3 genotype [26]. Interestingly, our study showed the proportion of the E2/E3 genotype was greater in the Type 2 DM without CAD group (12.8% versus 8.1%). However, data presented was based on limited sample size and it require further investigation to relate the association with risk of CAD. Most studies conducted in several other countries demonstrated that APOE ε4 allele increases the risk for CAD in Type 2 DM and non-diabetic patient, however current study does not demonstrate this association. Similarly, studies conducted in Slovenia [27], UK [28] and India also found there was no association between the APOE ε4 allele with CAD in Type 2 DM and non-diabetic patients [29].

Final objective of this study was to determine the association between APOE polymorphism with lipid levels in Type 2 DM without CAD and Type 2 DM with CAD groups. Patients were divided into 3 APOE subgroups which comprised of ε2, ε3 and ε4 alleles. Findings from this study demonstrated that Type 2 DM with CAD group had lower TC, TG and LDL-C compared to Type 2 DM without CAD group. These can be explained due to patients with diabetic complications tend to have more aggressive treatment and follow up. Further study on APOE polymorphism in the Type 2 DM with CAD group revealed there were no significant difference in all lipid parameters between ε2, ε3 and ε4 alleles. However, ε2 subgroup showed higher in median TG level and ε4 subgroup showed higher in median LDL-C level and lower in HDL-C. APOE is a ligand to LDL receptor. It is required for internally derived triglyceride rich lipoprotein metabolism which contain apoB-48. However, apoE2 is reported to be defective in binding to LDL receptor. This leads to type III dyslipoproteinemia among homozygous individuals [30]

Comparison in the Type 2 DM without CAD showed there were also no significant differences in TC, TG, HDL-C and LDL-C between ε2, ε3 and ε4 subgroups. In contrast to Type 2 DM with CAD, the ε4 subgroup showed higher TG level and lower level of HDL-C. As described by previous studies, ε2 carrier have lower TC and LDL-C, whereas carrier for ε4 allele have higher TC and LDL-C concentrations compared to those with the E3/E3 genotype. However, higher TG levels were found in both carrier of ε2 and ε4 alleles compared to E3/E3 individuals [31], [32]. Similarly, Jemaa et al. also showed that the ε2 carriers have lower TC and LDL-C, E3/E3 patients have intermediate concentrations and ε4 carriers have higher concentrations [33]. Findings from this study did not indicate significant association, however there was a trend particularly in ε4 allele subgroup having higher LDL-C and lower HDL-C level.

According to Malloy et al., increased in expression of the LDL-receptor (LDLR) is considered beneficial for reducing plasma cholesterol and atherosclerosis, as it would remove atherogenic lipoprotein particles from the circulation. High affinity of ε4 allele to the LDLR has been proposed to lead to increased APOE-mediated cholesterol uptake, higher intracellular cholesterol and thus a subsequent down-regulation of LDLR expression. Down-regulated LDLR level would then lead to a reduction in apoB100-mediated LDL clearance and thus high levels of circulating LDL cholesterol and an increase in atherosclerosis. This hypothesized down regulation of the LDLR by APOE4 has been thought to explain the association between apoE4 and increased risk of CVD [34].

Possible explanation to these less clear associations are due to effect of combination of particular genetic and environmental (lifestyle) factors. Distribution of the APOE alleles is widely variable, interaction between gender and furthermore APOE polymorphism has been reported to differ from one population to another [35], [36]. These observations, therefore, imply that the association of APOE polymorphism with the risk of CAD in diabetic and non-diabetic patients varies with populations.

V. Conclusion

This study demonstrates that the most frequent genotype was E3/E3 in both groups and was comparable with previous studies. However, there was no significant difference in APOE genotypes distribution in Type 2 DM without CAD and Type 2 DM with CAD group. Data also demonstrated that difference in APOE genotypes does not significantly influence lipid parameters, but there was trend indicating that ε4 subgroup gave rise to high LDL-C and lower HDL-C compared to ε2 subgroup, especially in Type 2 DM with CAD group. Nevertheless, the observed findings did not allow us to make a definitive statement about their association with the lipid parameters as it was not possible to establish any statistical relationship between the dyslipidemia and the genotype of APOE. This probably was due to limited sample size and the possible confounding factors including differences in the use of lipid-lowering agents and ethnicity in both groups.

Acknowledgment

This study was funded by Universiti Sains Malaysia RUI grant 1001/CIPPT/812138 and short term grant 304/PPSG/61312123. The authors would like to extend their appreciation to the editor and statistician.

References

[1] Sicree R, Shaw, J. & Zimmet, P: Prevalence and projections**.** *Diabetes atlas* 2006**:**16-104.

[2] Letchuman GR, Wan Nazaimoon WM, Wan Mohamad WB, Chandran LR, Tee GH, Jamaiyah H, Isa MR, Zanariah H, Fatanah I, Ahmad Faudzi Y: Prevalence of diabetes in the Malaysian National Health Morbidity Survey III 2006**.** *Med J Malaysia* 2010; 65**:**180-6.

[3] Cade WT: Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. *Phys Ther* 2008; 88:1322-35.
[4] Syvanne M, Taskinen MR: Lipids and lipoproteins as coronary risk factors in non-insulin-dependent diabetes mellitus. *Lancet* 1997; 350 Suppl 1:SI20-3.
[5] Chew BH, Ismail, M., Lee, P. Y., Taher, S. W., Haniff, J., Mustapha, F. I. & Bujang, M. A: Determinants of uncontrolled dyslipidaemia among adult type 2 diabetes in Malaysia: the Malaysian Diabetes Registry 2009. *diabetes research and clinical practice* 2012; 96:339-47. .
[6] Bennet AM, Di Angelantonio E, Ye Z, Wensley F, Dahlin A, Ahlbom A, Keavney B, Collins R, Wiman B, de Faire U, Danesh J: Association of apolipoprotein E genotypes with lipid levels and coronary risk. *JAMA* 2007; 298:1300-11.
[7] Damani SB, Topol EJ: Emerging genomic applications in coronary artery disease. *JACC Cardiovasc Interv* 2011; 4:473-82.
[8] Hixson JE, Vernier DT: Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *Journal of Lipid Research* 1990; 31:545-8.
[9] Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; 285:2486-97.
[10] Klein R: Hyperglycemia and microvascular and macrovascular disease in diabetes. *Diabetes Care* 1995; 18:258-68.
[11] Turner RC, Millns H, Neil HA, Stratton IM, Manley SE, Matthews DR, Holman RR: Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). *BMJ* 1998; 316:823-8.
[12] Standl E, Balletshofer B, Dahl B, Weichenhain B, Stiegler H, Hormann A, Holle R: Predictors of 10-year macrovascular and overall mortality in patients with NIDDM: the Munich General Practitioner Project. *Diabetologia* 1996; 39:1540-5.
[13] Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR: Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 2000; 321:405-12.
[14] Dyslipidemia Management in Adults With Diabetes. *Diabetes Care* 2003; 27:s68-s71.
[15] Mohamed E, Mohamed M, Rashid FA: Dyslipidaemic pattern of patients with type 2 diabetes mellitus. *Malays J Med Sci* 2004; 11:44-51.
[16] Cook CB, Erdman DM, Ryan GJ, Greenlund KJ, Giles WH, Gallina DL, El-Kebbi IM, Ziemer DC, Ernst KL, Dunbar VG, Phillips LS: The pattern of dyslipidemia among urban African-Americans with type 2 diabetes. *Diabetes Care* 2000; 23:319-24.
[17] Burman D, Mente A, Hegele RA, Islam S, Yusuf S, Anand SS: Relationship of the ApoE polymorphism to plasma lipid traits among South Asians, Chinese, and Europeans living in Canada. *Atherosclerosis* 2009; 203:192-200.
[18] Mamotte CDS, Burke V, Taylor RR, van Bockxmeer FM: Evidence of reduced coronary artery disease risk for apolipoprotein ϵ2/3 heterozygotes. *European Journal of Internal Medicine* 2002; 13:250-5.
[19] Hallman DM, Boerwinkle E, Saha N, Sandholzer C, Menzel HJ, Csazar A, Utermann G: The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. *Am J Hum Genet* 1991; 49:338-49.
[20] Muros M, Rodríguez-Ferrer C: Apolipoprotein E polymorphism influence on lipids, apolipoproteins and Lp(a) in a Spanish population underexpressing apo E4. *Atherosclerosis* 1996; 121:13-21.
[21] Chaudhary R, Likidlilid A, Peerapatdit T, Tresukosol D, Srisuma S, Ratanamaneechat S, Sriratanasathavorn C: Apolipoprotein E gene polymorphism: effects on plasma lipids and risk of type 2 diabetes and coronary artery disease. *Cardiovasc Diabetol* 2012; 11:36.
[22] Morbois-Trabut L, Chabrolle C, Garrigue MA, Lasfargues G, Lecomte P: Apolipoprotein E genotype and plasma lipid levels in Caucasian diabetic patients. *Diabetes Metab* 2006; 32:270-5.
[23] Seet WT, Mary Anne TJ, Yen TS: Apolipoprotein E genotyping in the Malay, Chinese and Indian ethnic groups in Malaysia-a study on the distribution of the different apoE alleles and genotypes. *Clin Chim Acta* 2004; 340:201-5.
[24] Vaisi-Raygani A, Rahimi Z, Nomani H, Tavilani H, Pourmotabbed T: The presence of apolipoprotein epsilon4 and epsilon2 alleles augments the risk of coronary artery disease in type 2 diabetic patients. *Clin Biochem* 2007; 40:1150-6.
[25] Wilson PW, Schaefer EJ, Larson MG, Ordovas JM: Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. *Arterioscler Thromb Vasc Biol* 1996; 16:1250-5.
[26] Koch W, Hoppmann P, Schomig A, Kastrati A: Apolipoprotein E gene epsilon2/epsilon3/epsilon4 polymorphism and myocardial infarction: case-control study in a large population sample. *Int J Cardiol* 2008; 125:116-7.
[27] Letonja M, Guzic-Salobir B, Peterlin B, Petrovic D: Apolipoprotein E gene polymorphism effects triglycerides but not CAD risk in Caucasian women younger than 65 years. *Ann Genet* 2004; 47:147-53.
[28] Hashim Y, Shepherd D, Wiltshire S, Holman RR, Levy CJ, Clark A, Cull AC: Butyrylcholinesterase K variant on chromosome 3 q is associated with Type II diabetes in white Caucasian subjects. *Diabetologia* 2001; 44:2227-30.
[29] Singh PP, Naz I, Gilmour A, Singh M, Mastana S: Association of APOE (Hha1) and ACE (I/D) gene polymorphisms with type 2 diabetes mellitus in North West India. *Diabetes Res Clin Pract* 2006; 74:95-102.
[30] Ginsberg HN: New Perspectives on Atherogenesis. *Circulation* 2002; 106:2137.
[31] Balcerzyk A, Zak I, Krauze J: Synergistic effects of apolipoprotein E gene epsilon polymorphism and some conventional risk factors on premature ischaemic heart disease development. *Kardiol Pol* 2007; 65:1058-65; discussion 66-7.
[32] Topic A, Spasojevic Kalimanovska V, Zeljkovic A, Vekic J, Jelic Ivanovic Z: Gender-related effect of apo E polymorphism on lipoprotein particle sizes in the middle-aged subjects. *Clin Biochem* 2008; 41:361-7.
[33] Jemaa R, Elasmi M, Naouali C, Feki M, Kallel A, Souissi M, Sanhaji H, Hadj Taïeb S, Souheil O, Kaabachi N: Apolipoprotein E polymorphism in the Tunisian population: Frequency and effect on lipid parameters. *Clin Biochem* 2006; 39:816-20.
[34] Malloy SI, Altenburg MK, Knouff C, Lanningham-Foster L, Parks JS, Maeda N: Harmful effects of increased LDLR expression in mice with human APOE*4 but not APOE*3. *Arterioscler Thromb Vasc Biol* 2004; 24:91-7.
[35] Gensini GF, Comeglio M, Colella A: Classical risk factors and emerging elements in the risk profile for coronary artery disease. *Eur Heart J* 1998; 19 Suppl A:A53-61.
[36] Joss N, Jardine A, Gaffney D, Boulton-Jones JM: Influence of apolipoprotein E genotype on progression of diabetic nephropathy. *Nephron Exp Nephrol* 2005; 101:e127-33.