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Sex Differences in the Regulation of Plasma Lipids by Apo E Phenotypes in a Group of Young Adolescents

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Abstract

Association between the lipid levels and restriction length polymorphism at the Apolipoprotein E (apo E) gene locus was studied in a sample of unrelated male (91) and female (65) adolescents whose mean age was 17. There was no significant variation in the distribution of the ε4, ε3 and ε2 alleles between the males (ε4 = 0.148, ε3 = 0.725, ε2 = 0.126) and the females (ε4 = 0.161, ε3 = 0.722, ε2 = 0.115). However the contribution of apo E polymorphism on the lipid level was different in both sexes. When the mean lipoprotein levels were compared between the males and females, the females had a significantly higher level of high density lipoprotein cholesterol (HDL-C) ($p = 0.008$) than the males. In the total number of adolescents used in this study, the E3/E2 phenotypes had a significantly ($p < 0.05$) lower level of total and low density lipoprotein (LDL) cholesterol than the E3/E3 and E3/E4 phenotypes. The E3/E4 phenotypes did not increase the level of total and LDL cholesterol. When the number of adolescents were divided into males and females, there were differences in the effect of apo E on the lipoproteins. In both males and females the E3/E2 group had a significantly lower level of total and LDL cholesterol ($p < 0.05$) than the E3/E3 and E3/E4 group. But the effect of the phenotypes on HDL cholesterol was different between the males and females. In the males the HDL-C in the E3/E2 group was significantly higher than the E3/E4 and the E3/E3 group ($p < 0.05$). In the females the HDL-C in the E3/E3 group was significantly higher than the E3/E4 and the E3/E2 groups, ($p < 0.05$). Our data show that the apo E phenotypes modulate the HDL-C differently in the males and females.

Introduction

Due to the increasing incidence of coronary heart disease (CHD) in Malaysia, this study, in a group of adolescents was aimed at identifying a genetic factor that may be related to its development. Apolipoprotein E phenotype has been proposed as an important genetic determinant of CHD, because of the association between apo E phenotypes and plasma total cholesterol (TC) and low density lipoprotein (LDL) cholesterol levels (Utermann *et al*., 1979; Robertson and Cumming, 1985). Apo E in plasma is mainly carried by chylomicrons , very low density lipoproteins (VLDL's) and high density lipoproteins (HDL). When associated with these lipoproteins, Apo E serves as a ligand for the LDL receptor and the LDL receptor related proteins on the surface of the hepatic cells (Mahley and Innerarity, 1983; Brenninkmeijer *et al*., 1987; Hussain *et al*., 1991). In humans , three common Apo E alleles are ε2, ε3 and ε4. The ε3 allele is probably the wild type and codes for the product designated E3. The ε2 allele codes for an Arg 158 Cys substituted product designated ε2, and the ε4 codes for a Cys 112 Arg substituted product E4 (Utermann *et al*., 1980; Zannis and Breslow ,1981). Since the alleles are expressed in a codominant fashion, six different phenotypes are expressed in the general population E4/E4, E3/E4, E3/E3, E3/E2, E4/E2 and E2/E2 (Zannis *et al*., 1982). Individuals with the 62 allele display low levels of total and LDL cholesterol, while 64 display high levels of total and LDL cholesterol (Sing and Davignon, 1985). However this has not been observed in all the populations studied so far, although diet, gender and ethnic origin have been put forward as influencing factors (De Knijff and Havekes, 1996). Some studies have also shown that apo E phenotype modifies the levels of high density lipoprotein cholesterol (HDL-C) and triglycerides in different populations (Braeckman *et al*.,1996; Dallongeville *et al*., 1992). It has also been demonstrated that the effect of Apo E phenotype on plasma LDL cholesterol is influenced by sex. Some studies on post menopausal women have suggested that sex hormones like eastradiol and testosterone can modulate the impact of apoE phenotypes on the levels of plasma lipids (Schaefer *et al*., 1994). Although sex remains a predictor of variations in HDL-C and coronary heart disease, there have been conflicting reports on how these hormones, modulate the impact of apoE phenotypes on the plasma lipid levels. Attention has been directed to sex differences in the high density lipoproteins (Kauma *et al*., 1996) and prospective studies have shown a strong predictive link between HDL cholesterol and ischaemic heart disease (Miller and Miller, 1975). Therefore further studies are necessary to evaluate the role of sex hormones on plasma lipids and their influence on apo E phenotypes. Our study was aimed at determining the influence of sex on the

effect of apo E phenotypes on the lipid levels in a group of Asian adolescents.

Materials and Methods

Subjects: One hundred and sixty adolescents consisting of Chinese, Malays and Indians were recruited from various public schools from Klang valley and University of Malaya, Kuala Lumpur.The total population of 15-19 year adults in Klang valley are estimated around 80,000 by the bureau of statistics. One hundred and fifty six students participated in the study, at the rate of 97.5 percent. Consent forms were signed by the parents before the start of the study. The protocol was approved by the ethics committee of the University Hospital, Kuala Lumpur, Malaysia for research involving human subjects. Females between the ages of 16-17, who were not pregnant, not on contraceptives and who had no history of high blood pressure, heart disease and diabetes, and males between the ages of 16-17 who had no history of blood pressure, heart disease and diabetes were eligible for this study.

Biochemical determinations: Blood was collected in bottles containing disodium ethylene diamine tetra acetate dihydrate (EDTA), and the plasma was separated immediately by centrifugation at 3000 rpm for 15 minutes at 40°C. Total cholesterol, triglycerides and high density lipoprotein were determined using the individual biochemical kits supplied with Dimension® clinical chemistry system (Dods Behring. France), and the low density lipoprotein were determined by Friedewald equation (Friedewald *et al*., 1972) . The apo E genotypes were identified by restriction isotyping, (Hixson and Vernier, 1990), and the frequencies were determined by gene counting, e.g the frequency of ε2 = apo E 2/2 + apo E 3/2 + apo E 4/2)/total number of alleles. The Hardy Weinberg expectation of the distribution was calculated from the percentage distribution of the phenotypes (Guo and Thompson,1992) and a chi square test (x^2), with five degrees of freedom for the total population and eleven degrees of freedom for the males and females were used to test for equilibrium. Statistical analysis were performed using the statistical package SPSS 8.0 for Windows. An analysis of variance was used to determine whether the mean values of the lipoproteins were homogenous among the different phenotypic groups and between the different sexes after adjusting for race. A p value of <0.05 was used to identify statistical significance.

Results

The apo 8 allele frequencies in the total population were ε4 =0.153 ε3 = 0. 724 ε2 = 0.122 (Tablel), and the distribution of the phenotypes between the observed values and the expected values were not significantly different (X^2 = 6.044, df = 5. $p>0.05$). The distribution of the ε4, ε3 and ε2 alleles in the males (0.148, 0.725, 0.126), and females (0.161, 0.722, 0.115) are shown in (Table 2). The distribution of the phenotypes were not significantly different (x^2 = 7.54,df =11, $p>0.05$) between the males and females. Another series of analysis examined the influence of apo E polymorphism on various lipoprotein parameters after grouping the subjects as E2 carriers, (genotypes ε3/ε2 plus ε2/ε2), E3 homozygotes (ε3/ε3), and E4 carriers (ε3/ε4 and ε4/84 group). The ε4/ε6 group was not included in the analysis of the lipoproteins because of the opposite effects of ε2 and ε4 alles on the lipoproteins. In the total number of adolescents used in this study, the total and LDL cholesterol were significantly lower in the E3/E2 phenotypes ($p<0.05$), than in the E3/E4 and the E3/E3 phenotypes (Table3). The E3/E4 group did not have a cholesterol raising effect . However the contribution of apo E polymorphism on the lipid level was different in both sexes. When the mean levels of the lipids were compared between the males and females (Table 4) the HDL-C was significantly higher (p = 0.008) in the females than the males. In both sexes the total and LDL cholesterol were significantly ($p<0.05$) lower in the E3/E2 group than in the E3/E3 and E3/E4 groups. In the males the HDL-C was significantly higher in the E3/E2 group than in the E3/E4 and E3/E3 group ($p<0.05$) (Table 5), whereas in the females the HDL-C was significantly higher ($p<0.05$) in the E3/E3 than in the E3/E4 group (Table 6).

Table 1: Observed and expected frequencies of apo E Phenotypes (%) and apo E allele in Malaysian adolescents (n = 156)

Phenotypes	Observed Frequencies	Expected Frequencies[1]
E4/E4	0.0	2.37
E3/E4	26.3	22.30
E4/E2	4.5	3.75
E3/E3	50.0	52.50
E3/E2	18.6	17.60
E2/E2	0.6	1.48
Alleles		
β4	0.153	
β3	0.724	
β2	0.122	

[1]Under hardly weinberg equilibrium, x^2 = 6.044, df = 5, $p>0.05$ shows no significant variation.

Discussion

The important finding in our study is that the HDL cholesterol was significantly higher in the females than in the males during adolescent years. A study done in Britain showed that adolescent girls had higher levels of total cholesterol and HDL-C than the adolescent boys and that there was a pronounced fall in HDL-C in the boys during adolescence (Orchard *et al*., 1980). In another study it was shown that there was a fall in HDL-C with increasing sexual maturity among the adolescent boys (Anding *et al*., 1996). The effect of the apo E phenotypes on the lipids between

Table 2: Observed and expected frequencies of apoE phenotypes (%) and apo E allele frequencies in male and female adolescents (n = 156)

	Males (n = 91)		Female (n = 65)	
Phenotypes	Observed frequencies	Expected frequencies	Observed frequencies	Expected frequencies
E4/E4	0.00	2.20	0.00	2.61
E3/E4	24.20	21.50	29.20	23.40
E4/E2	5.49	3.75	3.08	3.73
E3/E3	50.50	52.60	49.20	52.30
E3/E2	19.80	18.30	16.90	16.70
E2/E2	0.00	1.60	1.54	1.33
Alleles	Males		Females	
ε4	0.148		0.161	
ε3	0.725		0.722	
ε2	0.126		0.115	

Under hardy weinberg equilibrium X^2 = 7.54, df = 11, p>0.05 shows no significant variations between the males and females.

Table 3: The effects of Apo E polymorphism on the lipoproteins in the total number of adolescents.

	Total	E3/E4	E3/E3	E3/E2
n	149	41	78	30
Total Cholesterol mmol/l		4.595 ± 0.118	4.445 ± 0.083	3.669 ± 0.176*
Log Triglycerides mmol/l		-0.0328 ± 0.075	-0.2020 ± 0.052	-0.3737 ± 0.111
HDL mmol/l		1.055 ± 0.038	1.015 ± 0.029	1.109 ± 0.073
LDL mmol/l		3.123 ± 0.086	3.085 ± 0.075	2.207 ± 0.167*

Results are adjusted ± Se (adjusted for race). *E3/E2 is significantly lower than E3/E3 and E3/E4 for total cholesterol and LDL cholesterol.

Table 4: Effect of apo E phenotypes on the lipoproteins in males and females.

	Males (n = 91)	Females (n = 65)	p-value
Total cholesterol mmol/l	4.228	4.453	0.099
HDL mmol/l	0.968	1.107	0.008
LDL mmol/l	2.892	2.986	0.466
Triglycerides (log) mmol/l	-0.134	-0.258	0.080

Results are the mean values for the lipoproteins for males and females (adjusted for race). *There was a significant difference between the males and the females for HDL-C (P = 0.008).

Table 5: The effect of ApoE phenotypes on plasma lipoproteins in males.

	E3/E4 n = 22	E3/E3 n = 46	E3/E2 n = 18
Total Cholesterol mmol/l	4.5700 ± 0.170	4.3800 ± 0.121	3.5100 ± 0.218*
Log Triglycerides mmol/l	-0.0882 ± 0.116	-0.1289 ± 0.083	-0.3485 ± 0.149
HDL mmol/l	0.8900 ± 0.071	0.9230 ± 0.051	1.16200 ± 0.091**
LDL mmol /l	3.2200 ± 0.159	3.0700 ± 1.113	2.0600 ± 0.206*

Results are means ± SE adjusted to race. Pairwise comparison by Berferroni. *E3/E2 is significantly lower than E3/E3 and E3/E4 for total cholesterol, LDL (p<0.05). **E3/E2 is significantly higher (p<0.05) for HDL-C than the E3/E3 and the E3/E4 groups.

Table 6: The effect of Apo E henotypes on plasma lipoproteins in females.

	E3/E4 n = 19	E3/E3 n = 32	E3/E2 n = 12
Total Cholesterol mm/l	4.62 ± 0.159	4.7000 ± 0.130	3.8300 ± 0.261
Log Triglycerides mm/l	-1539.00 ± 0.084	-0.2991 ± 0.069	-0.3990 ± 0.137
HDL mm/l	1.01 ± 0.064	1.1800 ± 0.053**	1.0600 ± 0.106
WI mm/l	3.26 ± 0.160	3.1700 ± 0.127	2.3500 ± 0.25*

Results are means ± SE adjusted for race. *E3/E2 is significantly lower than E3/E3 and E3/E4 for total and LDL cholesterol (p<0.05). **E3/E3 is significantly higher than E3/E4 for HDL (p<0.05).

the males and females were only different for HDL-C. The E3/E2 phenotype had a significantly lowering effect on the total cholesterol, and LDL cholesterol in both sexes, when compared to the E3/E3 phenotypes. The apo E3/E4, however did not have a cholesterol raising effect when compared to the E3/E3 phenotypes in both sexes as expected of E3/E4 phenotypes, (Hallman *et al*., 1991). Similar effect was seen in a Turkish study (Mahley *et al*., 1995), where the E3/E4 had no cholesterol raising effect. A number of other investigations using a variety of study designs have shown that the triglyceride is also a risk factor for CHD specifically in women (Melissa ,1991) , but we did not see any significant differences in the triglyceride levels in both sexes in the different phenotypic groups. In the males the HDL-C in the E3/E2 group was significantly higher ($p<0.05$) than in the E3/E3 and E3/E4 groups. In the females , the HDL-C was significantly higher ($p<0.05$) in the E3/E3 group than in the E3/E4 group. Low plasma levels of HDL-C is associated with increased coronary heart disease (Gordon *et al*., 1977). The Helsinki Heart study showed that the mean increase of 11 per cent in HDL cholesterol was associated with a 34 per cent reduction in coronary heart disease (Manninen *et al*., 1988). The protective effect of "Femaleness" may be due to high HDL-C which is influenced by estrogen, (Cobb *et al*., 1992). Although sex remains an important predictor of variations in HDL-C and coronary heart disease, there is evidence that factors other than sexual maturation may be responsible for a fall in HDL-C levels. A study on a group of male Pima Indians aged 10-22 years failed to show any association between HDL cholesterol and estradiol/testosterone ratio (Bennion *et al*., 1978). Previous studies have assessed selected CHD risk factors in children and adolescents but there is only one study in Finland (Lehtimaki *et al*., 1995) that has assessed the lipid levels in relation to apo E and gender interaction in adolescents. Our results provide support for the hypothesis that apo E phenotypes modulate the lipoproteins differently between the males and females and that this difference is already apparent in the young adolescents.

In conclusion our data suggest that the apo E3/E2 phenotypes do not only decrease the risk of cardiovascular disease by decreasing the levels of total and LDL cholesterol but also by mediating high levels of HDL-C cholesterol. Despite the limitation of the cross sectional analysis, our study has provided information on the interrelationship between sex, lipids and apo E phenotypes in a group of young Asian adolescents. Further studies are necessary to evaluate the interaction of sex hormones, apo E phenotypes with the other cardiovascular factors on the lipid levels.

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