NO EVIDENCE OF ASSOCIATION BETWEEN APO E POLYMORPHISM AND BMI AND LIPIDS PROFILE IN TURKMEN POPULATION

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ABSTRACT

Apolipoprotein E (apoE) is a glycoprotein that plays an essential role in lipid transport and metabolism.The present study purposed to determine the distribution of apoE alleles in a Turkman population and its association with lipid profile. In a cross-sectional study 354 healthy Turkman were recruited. Fasting blood samples were collected for biochemical tests and DNA extracting. Genomic DNA was amplified using PCR-RFLP method. The resulting fragments of PCR product digestion were detected by polyacrylamide gel electrophoresis. This study showed that ε3 allele and E3/E3 genotype are the most common allele and genotype, while ε2 and ε4 alleles had lower frequencies, respectively. Two homozygote genotypes E2E2 and E4E4 were not detected in our sample. Results didn't confirm any association between apo E polymorphism with lipids concentration in Turkmen population.

Keywords: Apolipoprotein E; Polymorphysm; Genetics; Lipids.

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INTRODUCTION

Coronary artery disease and stroke are leading causes of morbidity and mortality worldwide (Lopez et al. 2006). Some metabolic factors such as elevated levels of fasting plasma triglyceride (TG) and total cholesterol(TC), abnormally low high-density lipoprotein (HDL-C) levels and elevated low-density lipoprotein (LDL-C) concentrations are well-established risk factors for atherosclerosis and coronary heart disease (CHD) (Vanuzzo et al. 2008; Kim et al. 2005; Hokanson et al. 1996). It is also well known that blood lipid concentration is determined by both environmental and genetic factors. ApoE is an important structural constituent of several serum lipoprotein classes that its genetic variation can affect its anti atherosclerotic effects (Davignon et al. 1999). Apolipoprotein E (apoE) is a 34 KD glycoprotein with 299 amino acids that is mainly synthesized in liver. Apo E has an essential role in the lipid metabolism via its role in lipid transport. (Ordovas et al. 2000; Eichner et al. 2002; Dallongeville et al. 1992).

The common variants of apo E polymorphism include major alleles ε2, ε3, and ε4 located on chromosome 19 and six corresponding genotypes (E3/E3, E3/E4, E2/E3, E4/E4, E2/E4, and E2/E2) (Das et al. 1985; Zannis et al. 1982).

Previous studies have shown that these three alleles have quantitative effects on lipid and lipoprotein levels. ε2 allele is associated with low levels of total cholesterol (TC),low density lipoprotein (LDL-C), and apoprotein B (apo B), whereas ε4 allele has correlation with the opposite effects (Davignon et al. 1988; Eichner et al. 2002; Braeckman et al. 1996). In some studies, a positive association has been showed between ε2 allele and serum concentrations of HDL-C (Mahley et al. 2000). Apo ε3 is the most frequent allele in all studied populations (Mahley et al. 2000).

In study of 198 healthy candidates in Southern Iran, frequencies of apoE alleles were: 6.3, 88.6 and 5.1% for ε2, ε3 and ε4, respectively (Bazrgar et al. 2008). In another study of 198 healthy Kurdish in west of Iran, frequencies of apoE alleles were: 6.66, 87.87 and 5.45% for ε2, ε3 and ε4, respectively (Vaisi-Raygani et al. 2007). The aim of present study was to determine apo E gene polymorphism and evaluate its effect on plasma lipids in Turkmen population in east north of Iran.

MATERIALS AND METHODS

1-Studied population

In a randomly sampled cross-sectional study of 354 healthy Turkman, referred to Health Center Lab for premarried tests in 3 cities (Gonbad, Kalale, Azadshahr) located in North Eastern Iran, South East of the Caspian Sea were recruited in this study in period of May to August 2011. All selected subjects Have had at least two generations from "Turkman" background. The inclusion criteria were all of subjects who interest to enroll in the study. The history of medication affected lipid metabolism determined as exclusion criteria. After obtaining informed consent, a checklist containing personal and demographic characteristics was completed for each patient. Height, weight and blood pressure were measured and recorded. Body Mass Index (BMI) was calculated based on the following formula; body weight /square height (kg/m2).

Fasting venous blood was collected from each subject, 2 ml in tubes with EDTA for DNA extracting and 5 ml without anticoagulant for biochemical tests. After centrifugation and separation, all samples were stored at -70 ℃ until analysis.

2-Laboratory methods

Serum glucose, total cholesterol, HDL-C, and triacylglycerol concentrations were measured using enzymatic colorimetric method by using Pars Azmoon kits (Pars Azmoon Co., Tehran, Iran). LDL-C concentration was performed using the Friedewald equation.

3-ApoE genotype determination

To extract Genomic DNA from the peripheral blood leukocytes, standard kit (Diatom DNA prep 100 – Genfanavaran co., Tehran) was used. Quality and quantity of extracted DNA was determined by spectophotometric and agarose gel(0.8 %) electrophoresis techniques.

4-PCR method

A 224bp fragment of this gene was amplificated using polymerase chain reaction (PCR) method. To optimize PCR condition actors including annealing temperature, number of cycles, step durations and DNA concentration were altered. PCR reaction contained 3µl DNA(75ng/ µl), 7.5 µl master mix, 0.5 µmol of each primer and some deionized water up to final volume of 15 µl. Sequence of used primers, suggested by Yin et al (2008)are showed in table 1.

Because of low quality DNA, PCR regime included 2 consecutive programs as presented in table 2. Electrophoresis on 2.5% gel was used to confirm authenticity of PCR product.

Table 1- Sequence of used primers for amplificating the investigated gene

Forward:	5'-ACAGAATTCGCCCCGGCCTGGTACAC-3'
Reverse:	5'- TAAGCTTGGCACGGCTGTCCAAGGA-3'

Table2- PCR program used in this study

Steps	Temp.(°C)	Duration	Num. of cycles
-Primary denaturation of DNA	95	5'	1
-Denaturation of DNA	95	65''	
-Annealing	61.8	65''	24
-Synthesis	72	90''	
-Denaturation of DNA	95	60''	
-Annealing	61.8	60''	26
-Synthesis	72	90''	
-Final synthesis	72	8'	1

5-Digesting Reaction

After PCR , 5 µl of the amplificated fragment was digested by 10 units of HhaI endonuclease enzyme following the factory protocol as showed in table 3. Samples were incubated for 16 hours at 37°C. Digestion products were run on 6% polyacrylamide gel in (60V/160Min) electrophoresis and then were stained using optimized silver staining method (Benbouza et al. 2006) as presented in table 4.

Table 3- Protocol of digesting APO E gene

Content		Used concentration	Volume
Enzyme	(10U/ µl)	10U	0.1µl
Buffer		10X	2 µl
PCR	product	-	5 µl
H2O		-	2.9 µl

Total=10

Table 4- Steps of fast silver nitrate staining method

Step	solution	duration(Min)
1-Fixation (100CC)	1CC ethanol 100%+ 100µl acetic acid+H2O	5
2-Washing	Distilled H2O	2
3- Staining (100CC)	Nitrate silver (0.1 g)+ H2O	10
4-Washing	Distilled H2O	2
5- Developing (100CC)	NaOH(1.5g)+ Formaldehyde 500µl + H2O	15-20
6-Washing	Distilled H2O	2

6-Genotyping method

Determining genotype of the investigated groups was performed based on presented information in table 5.

Table 5- Size of digestion reaction concerning to different genotypes of APO E gene

Genotypes	Fragment size(bp)
E2E3	91 , 83 , 48 , 38 , 35
E2E4	91 , 83 , 72 , 48 , 38
E3E3	91 , 48 , 38 , 35
E3E4	91 , 72 , 48 , 38

7-Statistical analysis

Strength of association between different variables and Apo E gene polymorphism was estimated by Chi-square and logistic regression using SPSS software, version 16. P ≤ 0.05 was considered as significant in statistical analysis.

RESULTS

344 subjects were participated in this study. The mean age of them was 27.8 ± 8.4 years. 35 % (n=121) of them were male. Neither of them gets medications affected on glucose or lipid metabolism. Baseline characteristics of participants have shown in table 6. The mean of TG in men (99.43 ± 99.32) was significantly higher than women (79.03± 50.19). The level of HDL cholesterol in females was higher than males (P< 0.0001).

Table 6: Basic characteristic and biochemical indexes of participants (n= 344)

Index	Mean ± SD			p-value
	Total (n=344)	Male (n=121)	Female(n=223)	
Age (yr)	27.83 ± 8.36	28.36 ± 8.35	27.56 ± 8.38	0.396
BMI (kg/m2)	25.02 ± 6.88	24.77 ± 8.06	25.16 ± 6.17	0.284
TG (mg/dl)	86.22 ± 71.99	99.43 ± 99.32	79.03± 50.19	0.001
T Chol (mg/dl)	179.30 ± 54.27	172.30 ± 52.77	183.11 ± 54.81	0.076
HDL (mg/dl)	35.40 ± 8.53	31.67 ± 8.14	37.44 ± 8.06	0.0001
LDL (mg/dl)	126.65 ± 52.11	120.74 ± 51.71	129.87 ± 52.16	0.12

BMI: Body Mass Index, **TG**: Triglyceride, **TChol**: Total Cholesterol, **HDL**: high density lipoprotein, **LDL**: low density lipoprotein. Independent sample T. test was used as analytic test to compare means between two sexes.

The distribution of Apo E genotypes and alleles is shown in Table 7. As shown in table 7, the most frequent genotype is E3E3 and allele is ε3. Frequencies of ε2 and ε4 were 11.63 and 6.83% respectively. Two homozygote genotypes of E2E2 and E4E4 were not detected in our samples. As shown in table 8, there is no statistically significant difference in BMI and lipid concentration between different genotypes of apo E.

Table 7: Distribution of Apo E genotype frequencies and alleles

Genotypes distribution N = 344							Alleles n = 688		
E4E4	E3E4	E3E3	E2E4	E2E3	E2E2	ε4	ε3	ε2	
n	0	39	225	8	72	0	47	561	80
%	0	11.34	65.41	2.32	20.93	0	6.83	81.54	11.63

Table 8: The mean of BMI and lipids concentration in different genotypes

Genotypes	N	BMI(kg/m2) Mean± SD	TG (mg/dl) Mean± SD	Chol. (mg/dl) Mean± SD	HDL (mg/dl) Mean± SD	LDL (mg/dl) Mean± SD
E2E4	8	25.46±5.38	80.19 ±52.00	167 ± 55.92	30 ±7.87	120.96±50.69
E2E3	72	24.39±5.30	85.98 ±49.24	187.22±55.21	36 ±9.4	134.02±52.51
E3E3	225	24.82±5.89	86.24 ±82.19	175.65±54.62	35.36±8.3	123.03±52.61
E3E4	39	26.18±12.48	91.12 ±49.61	188.55±51.43	35.84±8.7	134.49±50.69
Total	344	24.90±6.83	86.59 ±72.42	179.33±54.52	35.43±8.58	126.58±52.38
ANOVA (one way) P value		0.606	0.974	0.260	0.307	0.331

DISCUSSION

on the polymorphism of apoE in several populations have demonstrated the presence of three common apoE alleles, e2, e3, and ApoE epidemiologic studies are interesting researches regarding to its association with cardiovascular diseases. Studies e4, which code for genetic isoforms and determine six different phenotypes.

Our study is the first report of ApoE polymorphism in Turkmen sub-population Northern Iran. In our population, we observed that E3 allele was the most common, followed by the E2 and E4 alleles respectively. These results are in agreement with previous findings in Iran (Bazrgar et al. 2008; Tabatabaei-Malazy et al. 2012; Bazzaz et al. 2010). In some of previous reports the frequency of E2 allele was rarer than E4 (Burman et al. 2009; Zende et al. 2013; Al-khedhairy 2004).

E4E4 and E2E2 genotypes are rarer genotypes in all studies. In this study the frequency of ε2 allele was in high reported universal for human populations but homozygote E2E2 genotype was not seen in our population. Also the E4E4 genotype was not detected. It is an unexpected result because the intra-ethnic marriages are a tradition in Turkmen and we have awaited to detect these homozygote genotypes. The results of current study show no significant difference in TG, total cholesterol, LDL and HDL levels between different genotypes of ApoE. In reviewing the literature, several studies were found the association between ApoE genotypes and dyslipidemia as a important risk factor associated with atherosclerosis and coronary heart disease (Panza et al. 1999; Boulenouar et al. 2013).

In several studies Apo E4 allele has been linked to higher plasma lipids levels (Yin et al. 2013) and also has shown that the prevalence of the E4-containing phenotypes was significantly higher in subjects with cardiovascular and cerebrovascular diseases (Boulenouar et al. 2013; Haddy et al. 2002). In results of Zende et al study, was reported higher lipid level in group having E4 homozygous allele than other alleles (Zende et al. 2013). It is also demonstrated that the presence of Apo E2 can play a protective role against dyslipidemia 91 (Lehtinen et al. 1995; Mendes-Lana et al. 2007). A possible explanation for this difference might be that our samples were healthy and young. Moreover dyslipidemia is a multifactorial disorder, affected by genetic and environmental factors such as demographic characteristics, dietary habits, and lifestyle choices.

The current research was not specifically designed to evaluate environmental factors. Further experimental investigations are needed to determine the ApoE polymorphism in chronic diseases such as CHD, hyperlipidemia, obesity, diabetes, etc. Also Further research might investigate the role of environmental factors such as diet and physical activities in relation with ApoE polymorphism in pathogenesis of diseases related to serum lipids.

CONCLUSION

Our study showed that the ApoE epsilon polymorphism has not the expected impact on the plasma lipid profile in a Iranian Turkman population.However, due to the small sample size in most of the included studies and the selection bias existed in some studies, the results should be interpreted with caution.

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Competing interests

The authors declare that they have no competing interests

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