

Apo E polymorphism among Egyptian patients with essential hypertension

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Abstract Hypertension (HTN) is a chronic condition of concern due to its role in the causation of coronary artery disease (CAD), stroke, and other vascular complications. Essential hypertension (EH) is a multifactorial disorder arising from the influence of several susceptibility genes and environmental stimuli. Apolipoprotein E (Apo E) plays an essential role in clearance of chylomicron remnants and very low-density lipoproteins (VLDL). Apo E gene has three alleles (E2, E3, and E4) that give rise to six different genotypes. A significant association of E4 allele has been observed with HTN in addition to the other well-known risk factors and positive family history. Carriers of E4 allele form a higher risk group showing greater susceptibility to CAD. These observations emphasize the need of genotyping Apo E in patients with EH as an important molecular tool in personalized medicine. The aim of this work was to study the association between Apo E gene polymorphism and EH in Egyptian patients as well as correlating different Apo E genotypes with serum lipids. The study

was conducted on 50 patients with EH and 50 age-matched controls. DNA analysis was performed using polymerase chain reaction restriction fragment length polymorphism. The E3/E3 genotype was found in 85.42 % of patients, compared to 80 % in controls. E3/E4 (8.33 %) and E2/E3 (6.25 %) were lower in patients compared to controls 12 and 8 %, respectively. E4/E4 and E2/E2 genotypes were only found in two patients (4 %). Total cholesterol and low-density lipoprotein were significantly higher in E3/E4 as compared to E3/E3 and E2/E3. However, there was no significant difference in triglyceride, high-density lipoprotein, and VLDL.

Keywords Essential hypertension · Apolipoprotein E · Molecular · Genotyping

Introduction

Hypertension is one of the most common worldwide diseases affecting humans. It is an important public health challenge because of the associated morbidity. Hypertension (HTN) is a progressive cardiovascular syndrome arising from complex and interrelated etiologies. Early markers of the syndrome are often present before blood pressure elevation is sustained; therefore, HTN cannot be classified solely by discrete blood pressure thresholds. Progression is strongly associated with functional and structural cardiac and vascular abnormalities that damage the heart, kidneys, brain, vasculature, and other organs and lead to premature morbidity and death (Chobanian et al. 2003).

Essential or primary hypertension means that no medical cause can be found to explain the raised blood pressure with no underlying identifiable clinical indication, making it challenging for clinicians. Secondary hypertension which accounts for 5 to 10 % of all cases indicates that the high blood pressure is a result of another condition due to

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identifiable disorders in specific organs and tissues, such as kidney disease or tumors, for example, adrenal adenoma or pheochromocytoma (Pierdomenico et al. 2009).

Unfortunately, despite advances in understanding and treating HTN, its prevalence continues to rise. It is estimated that nearly one billion people across the globe (~26 % of the adult) are affected by HTN, and this figure could rise to 1.5 billion (~29 %) by the year 2025 as described by Kearney et al. (2005). In Egypt, it varies by region showing the highest prevalence (31 %) in Cairo as presented by Ibrahim (2001).

Apolipoprotein E (Apo E) is an Apo protein found in the chylomicron and intermediate density lipoproteins that binds to a specific receptor on liver cells and peripheral cells. Apo E was initially recognized for its importance in lipoprotein metabolism and cardiovascular disease. Also, it has been studied for its role in several biological processes not directly related to lipoprotein transport, including Alzheimer's disease, immune regulation, and cognition. Neonates with brain injuries and/or defects who also have abnormalities in the Apo E gene may have an increased risk for cerebral palsy (Singh et al. 2002). It is also an important determinant of intestinal cholesterol absorption (Kesäniemi et al. 1987) and plasma lipid levels (Davignon et al. 1988).

Genotyping of Apo E gene in patients with essential hypertension (EH) has become an important tool in personalized medicine as patients harboring the E4 alleles are at high risk of developing coronary artery disease and require special monitoring of lipid profile and blood pressure (Bhavani et al. 2005). The objective of the present work was to study the association between apolipoprotein E gene polymorphism and EH in Egyptian patients, correlating the different apolipoprotein E genotypes with serum lipid levels in order to understand the possible interaction between the specific genotype and the lipid profiles that can cause HTN.

Subjects and methods

Fifty Egyptian hypertensive patients from those attending Cairo University's Cardiology department were included in the study. Their age ranged from 40 to 70 years, with a male/female ratio of 1:3 and with the following inclusion criteria: SBP of ≥ 140 mmHg or DBP of ≥ 90 mmHg during two or more readings on two or more occasions and/or patients using antihypertensive agents. Exclusion criteria include patients with secondary HTN and those associated with renal, pulmonary, and Alzheimer's diseases.

Control group consists of participants aged 50 and sex matched with no family history of HTN or dyslipidemia. An informed consent has been obtained from all participants according to the National Research Centre Committee guidelines. All selected groups were subjected to full history taking, blood pressure measurement, and laboratory investigations including lipid profile assay and Apo E genetic study.

Specimen collection

Seven milliliters of 12-h fasting venous blood was withdrawn from each subject under complete aseptic conditions and divided into two tubes, 2 ml was collected in a plain tube and serum was separated for lipid profile assay while 5 ml was collected in a sterile EDTA vacutainer tube for DNA extraction for Apo E gene study. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations were determined enzymatically using commercially available kits on an autoanalyzer (Olympus AU400, USA). Low-density lipoprotein cholesterol (LDL-C) was estimated using Friedewald's formula (Friedewald et al. 1972).

Fig. 1 A 10 % polyacrylamide gel stained with ethidium bromide illustrating the digestion of PCR products of exon4 of Apo E gene with *HhaI* restriction enzyme. Lanes 1–5, 8, and 10 seven control subjects with E3/E3 genotype (91, 48, 43, and 35 bp). Lanes 6, 7, and 9 three control subjects with E3/E4 genotype (91, 72, 48, 43, and 35 bp). Lane 11 one control subject with E2/E3 genotype (91, 83, 48, 43, and 35 bp). M molecular weight ladder (25 bp)

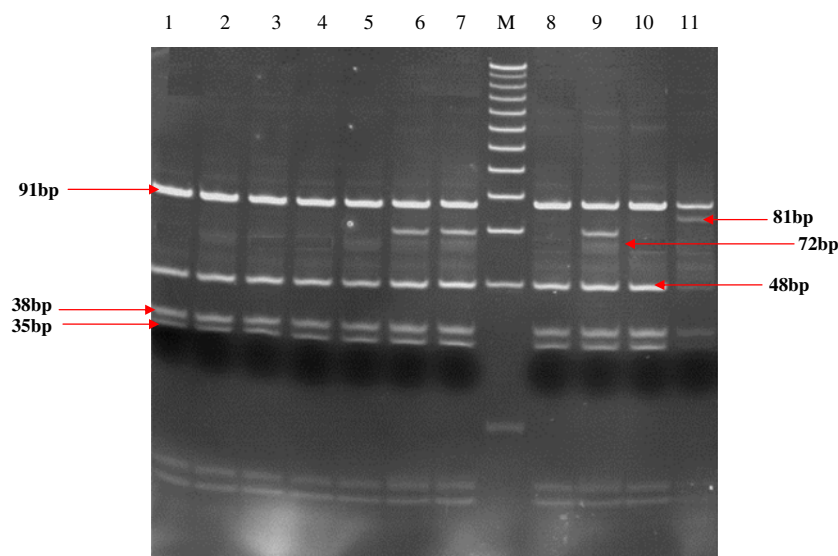


Table 1 Apo E genotypes in hypertensive patients and controls

	Control (<i>n</i> =50) <i>N</i> (%)	Patients (<i>n</i> =48) <i>N</i> (%)	<i>p</i> value
E3/E3	40 (80)	41 (85.42)	0.773
E3/E4	6 (12)	4 (8.33)	
E2/E3	4 (8)	3 (6.25)	
<i>p</i> value	0.00	0.00	

DNA isolation and Apo E exon4 genotyping

Genomic DNA was extracted from peripheral blood leukocytes of EDTA anticoagulated blood using salting out technique (Miller et al. 1988). Enzymatic amplification was performed by PCR using Taq polymerase enzyme and Bio-metra thermal cycler (GmbHm) Amplification of exon4 of the Apo E gene was performed using two primers (MWG Bio-technologies Eurofins MWG Operon, Anzinger Str. 7a, 85560 Ebersburg, Germany) as proposed by Li et al. (2003): forward primer 5'- ACAGAAATT GCC CCGGCCTGG TAC AC -3' and reverse primer 5'- TAAGCTTGACGGC TGTCCA AGG A -3'.

The PCR reaction mixtures were made to a total volume of 50 µl containing 0.5 µg genomic DNA, 10× buffer, 0.25 mM dNTPs, 2.5 pmol of each primer, and 2 units of Taq polymerase; reagents were supplied by Finnzyme (Finnzymes OY, Keilaranta 16 A, 02150 Espoo, Finland). The reaction was carried out with the following cycles: denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 64 °C for 1 min, and elongation at 72 °C for 2 min followed by a final elongation at 72 °C for 10 min. Detection of the amplified products in 2 % agarose gel containing ethidium bromide was done by performing E/P. The amplified products appeared as a single band of 244 bp. A 20-µl aliquot of each reaction was digested with 10 U of *Hha*I from Promega Corporation (2800 Woods Hollow Road Madison, W 153711–5399 USA) and electrophoresed on an 8 % polyacrylamide gel. Restriction digestion fragments were stained with ethidium bromide and photographed under ultraviolet light. Genotypes and alleles were determined using the method of Richard et al. (1994) (Fig. 1).

Table 2 Allele frequencies in hypertensive patients and controls

Variable genotypes	Patient (<i>n</i> =100)	Control (<i>n</i> =100)	<i>p</i> value
E2 allele	5	4	0.943
E3 allele	89	90	
E4 allele	6	6	

Table 3 Prevalence of Apo E genotypes in hypertensive patients and controls

Genotype	Patient (<i>N</i> =50)	Controls (<i>N</i> =50)	<i>p</i> value
E3/E3	41	40	0.635
E3/E4	4	6	
E2/E3	3	4	
E4/E4	1	0	
E2/E2	1	0	

Statistical methods

Data were expressed descriptively as percentages for qualitative values and mean±standard deviation (SD) for quantitative parametric data. The complied data were computerized and analyzed by SPSS software package version 2. The following tests of significance were used: analysis of variance test between more than two means and *t* test between means to analyze mean difference. Comparison of qualitative data was done using chi-square test, cross tabs, and LSD. A level of significance with $p \leq 0.05$ was considered significant; $p \leq 0.01$ was considered of high significance, and $p > 0.05$, insignificant.

Results and discussion

Analysis of data of this study revealed lower HDL-C and higher TG values in patients than in controls; however, the difference was not statistically significant. This study revealed no significant differences between TC, very low-density lipoproteins and LDL-C in patients compared to controls. This comes in contrast with the study performed by Bhavani et al. (2005) who revealed significantly elevated levels of TG in patients than in controls. Also, HDL-C levels were found to be significantly reduced in patients, while TC and LDL-C levels were significantly elevated in patients than in controls.

Apo E genotyping frequency in this study showed E3/E3 to be 85.42 % in patients compared to 80 % in controls; E3/

Table 4 Plasma lipids measurements in different Apo E genotypes

Variable	Mean ± SD			<i>p</i> value
	E3/E3 (<i>n</i> =81)	E3/E4 (<i>n</i> =10)	E2/E3 (<i>n</i> =7)	
Total cholesterol (mg/dl)	203.02±56.93	244.5±44.01	158.43±25.36	0.007**
Triglyceride (mg/dl)	134.59±102.11	158.5±93.04	119.71±35.07	0.695
HDL-C (mg/dl)	43.23±10.88	46.3±15.17	38.71±7.25	0.391
LDL-C (mg/dl)	134.1±50.81	166.3±48.87	93.14±24.4	0.013*
VLDL (mg/dl)	25.2±11.54	31.8±18.51	23.86±7.15	0.252

*Significant, **Highly significant

E4 (8.33 %) and E2/E3 (6.25 %) were lower in patients compared to controls, 12 and 8 %, respectively (Table 1). It is worth mentioning that the genotype E4/E4 and E2/E2 were not found in the control group and were only found in two patients (4 %). It was observed that the frequency of Apo E alleles was similar in both patients, and controls with no significant differences between the two groups were found, $p=0.943$ (Table 2).

In this study, E2/E3 was found to be 6.25 % in patients and 8 % in the control group; this comes in accordance to the study by Bhavani and coworkers (2005). Prevalence of E2/E3 genotypes was lower in patients than in controls, 4.3 vs. 6.5 %. E3/E4 was 8.33 % in patients and 12 % in controls. In contrast, Li et al. (2003) revealed that E3/E4 genotype frequencies were higher in the hypertensive patients than in controls (E3/E4 14.89 vs. 6.86 %).

Similarly, Al-Khedhairy (2004) showed in a study on 165 normal Saudis that the prevalence of genotypes E3/E3 and E3/E4 were found to be 71 and 27 %, respectively. However, other genotypes E2/E2, E2/E3, and E2/E4 were absent showing the absence of E2 allele (Table 3).

Allele frequencies in this study were: E2, 0.05 %; E3, 0.89 %; and E4, 0.06 %. In accordance to these results, Al-Yahyaee et al. (2005) showed that Apo E allele frequencies were: E2, 0.052 %; E3, 0.886 %; and E4, 0.062 % in a study performed on healthy Omani. The pattern of distribution, characterized by the lowest E4 and among the highest E3 allele frequencies, in the world, was very similar to that of Arabs, Southern Europeans of Mediterranean basin, Indians, and Japanese populations.

Results of this study showed that total cholesterol was significantly higher in E3/E4 (244.5 ± 44.01 , $p=0.007$) compared to 203.02 ± 56.93 and 158.43 ± 25.36 in E3/E3 and E2/E3, respectively, (Table 4). Also, LDL showed higher significance in E3/E4 (166.3 ± 48.87 , $p=0.013$) compared to 134.1 ± 50.81 and 93.14 ± 24.4 in E3/E3 and E2/E3, respectively. No difference between the three genotype groups was noted as regard to both systolic and diastolic blood pressures, $p=0.991$ and 0.866 , respectively.

Family history of EH was reported in 12/48 of patients (25 %, $p=0.000$) compared to 0 % in controls. Significant difference was found also in smoking which was reported in 4/48 (8.33 %, $p=0.037$) of hypertensive patients compared to 0 % in controls. In accordance to our study, 42 % of the patients had a positive family history for EH as reported by Bhavani et al. (2005), compared to 18 % positive family history among the controls, $p<0.05$.

There have been several studies of the association between the Apo E genotypes and prevalent HTN, with inconsistent findings. Results from small prevalent case–control studies have consistently described a positive relationship between the presence of the E4 allele and HTN or with greater BP levels (Li et al. 2003; Bhavani et al. 2005; Niu et al. 2007).

Some studies have suggested that high blood pressure may be associated with the presence of the E4 allele (Dembińska-Kieć et al. 1998; Yilmaz et al. 2001; Li et al. 2003), while others have found its association with E2 allele (Couderc et al. 1993). However, no association was found in few studies (Katsuya et al. 2002). In this study, only one male E4/E4 genotype had an elevated blood pressure with a history of ischemic heart disease and diabetes and was a heavy ex-smoker.

The results from cross-sectional studies have been more inconsistent. Four investigations carried out in mixed population of younger and older adults lack association between the Apo E genotype and HTN in USA (Wilson et al. 1994) and Tunisia (Jemaa et al. 2006), and a positive association between the E2 allele and prevalent HTN among male, but not female, Japanese males (Imazu et al. 2001). Also, a negative association between the presence of the E4 allele and the prevalence of HTN in the young, but not the old, was proposed by Katsuya et al. (2002).

In conclusion, this sample of Egyptian population showed no significant association between the Apo E gene polymorphism and EH. The distribution of the Apo E gene alleles is similar to that observed in most populations with a low prevalence of E4 allele. Further investigations involving wider test sample is warranted to confirm the role of Apo E gene polymorphism in the development of EH. Furthermore, the interplay between genetic and environmental factors must be thoroughly considered in order to evaluate the etiological role of Apo E polymorphism in HTN.

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