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Original Article

Endothelial nitric oxide synthase Glu 298 Asp (G894T) and *Apolipoprotein E* gene polymorphism as possible risk factors for coronary heart disease among Egyptians

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ABSTRACT

In Egypt, The prevalence of chronic heart disease (CHD) is 8.3%. It is the principal cause of death and is responsible for 22% of total mortality. The age-adjusted mortality rate is 174 per 100,000 of population. There are many studies on traditional risk factors and CHD in Egypt but the study of novel risk factors is deficient.

Objectives: The aim of the present case control study was to investigate the relation between CHD susceptibility and *Endothelial Nitric Oxide Synthase* (eNOS) Glu 298 Asp (G894T) and *Apolipoprotein E* (ApoE) gene polymorphism in a cohort of Egyptian individuals.

Methods: Genotyping of eNOS (Glu298Asp) and Apo E genes polymorphisms were done using polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method for 100 CHD cases and 100 age and sex matched healthy controls.

Results: A statistically significant association was observed between GT and TT genotypes of endothelial nitric oxide synthase gene with CHD with OR = 2.03 and 3.5; respectively. Also, carriers of E4 allele and especially E3/E4 genotype were at higher risk of CHD with OR = 3.3 for both. Significant association was also observed between the presence of combined GTE3E4 genotype and CHD with OR = 6.6.

Conclusion: GT and TT genotypes of endothelial nitric oxide synthase gene, E3/E4 genotype of Apo E gene polymorphism and combined GTE3E4 genotype can be considered risk factors for the development of CHD among Egyptians.

1. Introduction

In 2015, Cardiovascular diseases (CVD) have been considered the leading global causes of death with 20 million deaths accounting for 30% of all deaths worldwide, a number that is expected to increase to more than 23.6 million by 2030.[1]

In Egypt, The prevalence of coronary heart disease (CHD) is 8.3%. It is the principal cause of death and is responsible for 22% of total mortality. The age-adjusted mortality rate is 174 per 100,000 of population, ranking Egypt as number 33 in the world.[2]

CHD is a multifactorial disease, meaning that risk factors could be multiple, ranging from social, economic, psychological, lifestyle and biological. But continued focus on newer factors is warranted as they may improve our ability to predict future risk and determine treatment when they are included with the classical risk factors as genetic factors such as mutations at specific chromosomal locations and single nucleotide polymorphisms.[3]

Among these observed polymorphisms was replacement of glutamate by aspartate (Glu298Asp) or Guanine to thymine polymorphism at position 894(G894T) polymorphism of the human *endothelial nitric oxide synthase* (*eNOS*) gene.[4]

Nitric oxide (NO) is a potent vasodilator released by the endothelium and also by platelets and vascular smooth muscle cells. It plays important roles in protecting the cardiac vascular network against myocardial damage through inhibiting platelet aggregation, proliferation of vascular smooth muscle cells and leukocyte adhesion to the vascular endothelium.[5]

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Endothelial NO is synthesized by the enzyme eNOS that is encoded by the gene located on chromosome 7q35-q36. The (G894T) polymorphism of the *eNOS* gene had been studied and T allele had been described as susceptibility allele for CHD. There have been conflicting reports on the relationship between this polymorphism and CHD from studies done in various ethnic groups across the world.[6]

Another studied polymorphism called *Apo lipoprotein E* (*ApoE*) polymorphism had been found to be associated with CHD. Apo E is an essential part of lipoprotein metabolism which is present in lipoprotein particles and mediates lipoprotein binding to the LDL and lipoprotein remnant receptors. It is observed that defects in the Apo E protein (Apo E polymorphism) reduce its ability to bind to the receptors that leads to an elevated blood cholesterol level which is one of the major risk factors for CHD.[7]

The *ApoE* gene is located at chromosome 19q13.2 and 3 different alleles; E2, E3 and E4 account for *ApoE* polymorphism and determines the six genotypes; E2/2, E2/3, E2/4, E3/3, E3/4, and E4/4. CHD appears to be higher in the presence of the *ApoE*4 allele, and people with E4/E4 genotype are at a higher risk of developing the disease.[8]

Fortunately, the identification of genetic susceptibility traits will allow for more accurate risk stratification of patients. Hopefully, this will lead to the improvement of specific interventions that reduce the overall risk of CAD. This information that will be available at an earlier age will allow for the preventive measures to be applied earlier, and this is the cornerstone of personalized medicine.[9]

Although many candidate genes for CHD have been tested, the optimal set of risk genotypes has yet to be identified. Only a relatively modest risk can be expected in association with any single genotype and this risk increases with combined genotypes.[10]

Therefore, the aim of the present case control study was to investigate the relation between CHD susceptibility and Endothelial Nitric Oxide Synthase (eNOS) Glu 298 Asp (G894T) and Apolipoprotein E (ApoE) gene polymorphism in a cohort of Egyptian individuals.

2. Subjects and methods

2.1. Study population

A hospital based matched case control study was conducted in Mansoura University Hospitals in Egypt, during the period from August 2016 to August 2017. The study included a convenient sample of 200 subjects (100 cases and 100 controls):

Cases: Included newly-diagnosed cases of CHD. Patients with standard diagnostic criteria were recruited from ICU of cardiovascular department in Mansoura Specialized Medical Hospital.

Inclusion criteria for cases:

- The newly diagnosed patients with the first cardiac attack to avoid recall bias and change in behavioral risk factors of CHD.
- Fully conscious, co-operative, and well-oriented with time, place and person.
- All patients were from Egypt with both Egyptian parents.

Exclusion criteria for cases:

- Patients with previous myocardial infarction or previous revascularization.
- Patients with end stage renal disease.
- Patients with advanced liver cirrhosis.

Controls: A control was defined as age and sex matched subjects with no clinical evidence of CHD. They were recruited from other departments (such as ophthalmology, ENT, blood banks, and outpatient clinics).

Eligibility criteria for control: fully conscious, co-operative, and well-oriented with time, place, and person, who voluntary agree to participate in the study. Controls were selected to be matched with cases, ie, of the same sex and within ±3 years of age.

All controls were from Egypt with both Egyptian parents.

2.2. Study tool

An interviewer-administered structured questionnaire was done and including socio-demographic characteristics such as age, sex, residence, marital status, education, occupation, income.

Blood samples were collected from antecubital vein of both patients and control subjects between 8 and 10 a.m after a 12-h overnight fasting. Each sample was divided into two tubes, one EDTA tube and one glass tube; the sample in the glass tube was used for lipid profiling. The EDTA sample kept at −20 °C until use for genotyping.

Genotyping of *ApoE* and *eNOS* (Glu298Asp) gene polymorphisms:

Genomic DNA was extracted from whole venous EDTA blood using the GeneJET Whole Blood Genomic DNA Purification Mini Kits (Thermo Scientific, lot 00138029, Lithuania, EU) and stored at −20 °C until use. The genotypes of *ApoE* and *eNOS* Glu298 Asp SNPs were analyzed by the polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method according to Marrzoq et al.[8] for *ApoE* and Salimi et al.[11] for *eNOS* Glu298Asp.

Genomic DNA from the cases and controls was subjected to PCR analysis of the *ApoE* and *eNOS* genes using the following primers: *ApoE*; forward primer 5′-TCC AAG GAG CTG CAG GCG GCG CA-3′, reverse primer 5′-GCC CCG GCC TGG TAC ACT GCC A-3′; *eNOS*; forward primer 5′-GAC CCT GGA GAT GAA GGC AGG AGA-3′ and reverse primer 5′-ACC TCC AGG ATG TTG TAG CGG TGA-3′.

Reaction volume was 25 μl: 5 μl DNA at 100 ng/μl, 15.0 μl DreamTaq Green PCR master mix (Fermentas, Germany), 0.5 μl of each primer (25 pmol/μl) , and 4.0 μl H2O. Reaction conditions were carried out in thermocycler PTC-100 (Biorad, USA) with the following cycling parameters. For *ApoE*, the PCR conditions included an initial 95 C for 3 min followed by 40 cycles of 95 C for 60 s, 58 C for 60 s, and 72 C for 90 s and a final extension at 72 C for 10 min. For *eNOS*, the PCR conditions included an initial 94 C for 5 min followed by 30 cycles of 94 C for 30 s, 61 C for 30 s and 72 C for 30 s and a final extension at 72 C for 6 min. 10 μl of PCR products were resolved in 2% agarose gel to check the PCR products at 218 bp for *ApoE* and 517 bp for eNOS.

Restriction fragment length polymorphism (RFLP) analysis was done using FastDigest AflIII for *ApoE* (lot number 00125959) and BanII for *eNOS* (lot number 00136799) (Fermentas, Germany). 30 μl total volume reaction was prepared by mixing: 10 μl of PCR products + 1.0 μl of restriction enzyme + 2.0 μl 10× FastDigest green buffer +17 μl nuclease-free water. The mixture was incubated at 37 C for 10 min followed by heating at 65 C for 10 min. DNA fragments were resolved in 2.5% agarose gels. Genotypes were determined as follow; ApoE polymorphism: E3 = 145 bp fragment, E2 = 168 bp fragment, E4 = 195 bp fragment; eNos polymorphism: The wild-type allele (G) has no *BanII* cleavage site, whereas the PCR product was cleaved into two fragments of 346 and 171 bp in the presence of the T984 Figs. 1 and 2.

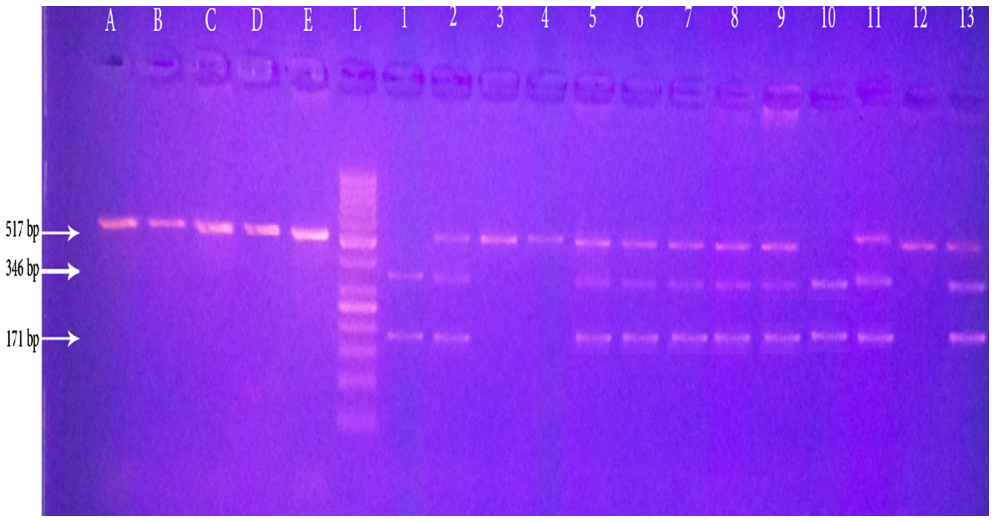

Fig. 1. Of Enos amplification product, after digestion with restriction enzyme Ban II. Lanes (A, B, C, D, E): 517pb Enos PCR product, lane (L): 50 pb ladder . Lanes (1, 10): homozygous individuals for T alleles yielded two fragments of 346pb and 171pb. Lanes (2, 5, 6, 7, 8, 9, 11, 13): hererozygous individuals for G and T alleles yielded 3 fragments of 517pb, 346pb and 171pb. Lanes (3, 4, 12): homozygous individuals for the G alleles resulted in no cleavage (517 pb).

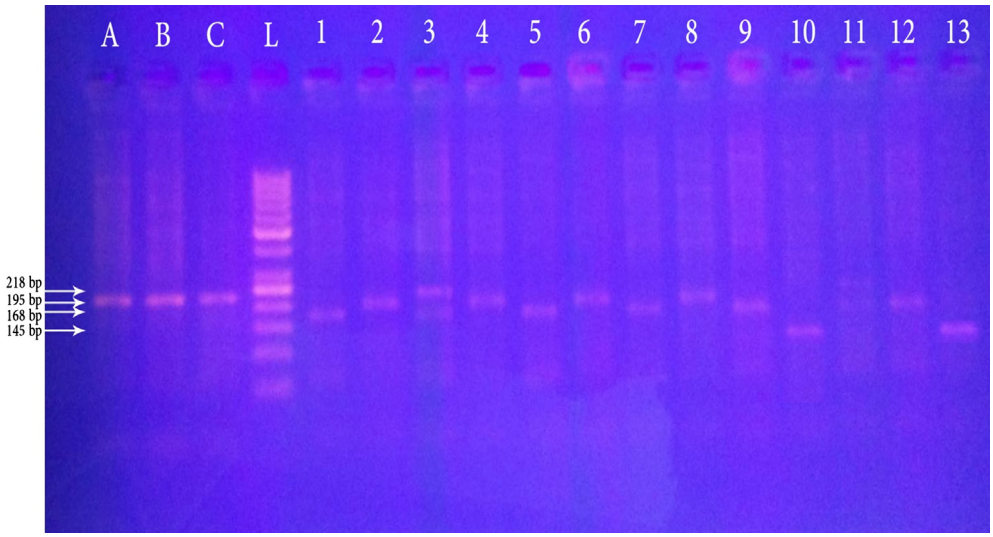

Fig 2. Of Apo E amplification product, after digestion with restriction enzyme AflII, Lanes (A, B, C): 218pb PCR products, Lane (L): 50 pb ladder. Lanes (1, 5, 7.9, 12): 168 pb for E2 allele. Lanes (2, 4, 6.8):195 pb for E4 allele. Lanes (10, 13): 145 foe E3 allele.

2.3. Ethical consideration

- Subjects gave their consent to participate in the study. All the information that was obtained about the subjects was kept confidential.
- Study protocol was approved by Institution Research Board (IRB) of Mansoura medical college.

2.4. Data analysis

Data were entered, cleaned to identify inconsistencies and statistically analyzed using the Statistical Package for Social Science (SPSS) version 16. The normality of data was first tested with one-sample Kolmogorov-Smirnov test. Qualitative data was described using number and percent. Association between categorical variables was tested using Chi-square test. When more than 25% of the cells have expected count less than 5, Fisher's exact test or Monte Carlo test were used, as appropriate. Continuous variables were presented as mean ± SD (standard deviation) for parametric data and median for non-parametric data. Independent sample *t*-test was used to compare means (parametric data) while Mann Whitney test was used for comparison of median (non-parametric data). Deviations from Hardy–Weinberg equilibrium expectations were determined using the chi-squared test at a significance level of $P < 0.05$.

3. Results

Table 1 showed that both cases and control groups were matched regarding all their socio-demographic characteristics. Baseline characteristics of CHD patients are summarized in Table 2.

There was a significant difference between CHD patients and healthy control in the allelic distribution of the eNOS ($P = 0.002$) and Apo E polymorphisms ($P = 0.02$). Also with Bonferroni adjustment, The significance was found, Therefore, the T allele of eNOS and E4 allele of Apo E were higher in CHD patients than controls suggesting that these alleles may demonstrate a susceptibility

Table 1
Socio-demographic features of cases versus controls.

Socio-demographic characteristics	Cases = 100 (%)	Controls = 100 (%)	Significance test	OR(95%CI)
Age				
<50 ys (r)	28	29		1
50−ys	36	35	$\chi^2 = 0.03, \prod = 0.86$	1.07 (0.5–2.3)
60+ys	36	36	$\chi^2 = 0.01, \prod = 0.92$	1.04 (0.5–2.2)
Min-Max	28–75	30–75		
Mean ± SD	55 ± 9.9	54.7 ± 9.7	t = −0.2, P = 0.84	
Sex				
Female (r)	15	15	Not applicable	Undefined
Male	85	85		
Residence				
Rural(r)	39	38		1
Urban	61	62	$\chi^2 = 0.02, \prod = 0.88$	0.96 (0.02–1.7)
Education				
Illiterate/Read and write(r)	41	39		1
Secondary or less	48	46	χ^2	0.99 (0.52–1.8)
More than secondary	11	15	χ^2	0.7 (0.26–1.8)
Occupation				
Non-working/housewife(r)	12	6		1
Manual /Farmer/Trades	51	51	χ^2	0.5 (0.15–1.5)0.43 (0.13–1.4)
Semi prof/Professional	37	43	χ^2	
Income				
Sufficient (r)	11	19		1
Insufficient	89	81	χ^2	1.9 (0.8–4.5)

r: reference group.

Table 2
Clinical and laboratory characteristics of CHD patients.

Patients (n = 100)	Value
Chest pain: Retrosternal/Epigastric	91/9
Radiating pain: No/Yes*	24/76
Site of radiation (N/%)	
Left shoulder.	59 (77.6)
Right shoulder	5 (6.6)
Left arm	25 (32.9)
Right arm	4 (5.3)
Back	46 (60.5)
Nature of pain	
Stabbing/Burning/Compressing	52/25/23
Precipitating factors: (N/%)	
Stress	3 (3)
Heavy work	22 (22)
Heavy meal	12 (12)
During quite sleep	58 (58)
During setting	5 (5)
Duration of pain (Mean ± SD)	3.4 ± 1.165
ECG findings; (N/%)	
STEMI	68 (68)
NONSTEMI(T wave inversion)	22 (22)
NONSTEMI (ST depression)	10 (10)
ECHO finding	
Ejection fraction(Mean ± SD)	54.7 ± 4.7
Segmental wall motion: (N/%)	
Anterior wall MI	45 (45)
Inferior wall	29 (29)
Lateral wall	26 (26)
Lipid profile: (N)	
Cholesterol (mg/dL): <200/≥200	19 / 81
TG (mg/dL): <150/≥150	33 / 67
LDL(mg/dL): <130/≥130	28/72
HDL(mg/dL): >45/≤45	65/35

* Categories are not mutually exclusive. (Percent calculated from patients with radiating pain).

effect to CHD in our cohort (OR = 1.9 (95% CI = 1.2–3.1) and 3.3 (95% CI = 1.02–10.9)., respectively) as shown in Tables 3 and 4.

Analysis of the genotype distribution between CHD patients and controls showed A statistically significant association was observed between GT and TT genotypes of endothelial nitric oxide synthase gene with CHD with OR = 2.03(95% CI = 1.07–3.8) and 3.5 (95% CI = 1.1–11.2); respectively.

Also, an increased frequency of E3/E4 genotype of Apo E gene in CHD patients was found, and the difference was statistically significant (P = 0.02), with Bonforroni correction (Pc = 0.04). The presence of E3/E4 genotype increases the risk of CHD 3.3 fold (95% CI, 1.02–10.94).

The distribution of genotypes for both polymorphisms in control group did not differ significantly from that expected in the general population under Hardy-Weinberg equilibrium, (P > 0.05) as shown in Tables 3 and 4.

When analyzing the combined genotypes of the two studied polymorphisms, significant association was observed between the presence of GTE3E4 genotype and CHD ($P \leq 0.001$) as the presence of GTE3E4 genotype increases the risk of CHD 6.6 fold (95% CI = 1.7–29.5) as shown in Table 5.

It was found that both eNOS and ApoE genotype polymorphisms were not associated with any of the clinical or laboratory parameters of CHD patients as shown in Tables 6 and 7.

The similarities in eNoS alleles' frequencies with the current study were compared with previous studies from multiple countries using pair wise fixation index (FST). Moderate genetic differentiation was found with Yoshimura et al.[28], and Shimasaki et al.[29] from Japan, while little genetic differentiation was found when comparing the current study versus the rest of studies in Table 8. Colombo et al.[44] study from Italy was excluded from comparison as they were not in HW equilibrium. Also, the similarities in ApoE alleles' frequencies with the current study were compared with previous studies from multiple countries. Moderate genetic

Table 3
Distribution of *eNOS* (Glu298Asp) alleles and genotypes in CHD patients and healthy controls.

eNOS Polymorphism	CHD Patient (N = 100) N/%	Controls (N = 100) N/%	OR (95% CI)	P/Pc
Alleles (n = 200)				
G	126/63	154/77	1	
T	74/37	46/23	1.9 (1.2–3.1)	0.002/0.004*
Genotypes				
GG	40/40	60/60	1	
GT	46/46	34/34	2.03 (1.07–3.8)	0.01/0.02*
TT	14/14	6/6	3.5 (1.1–11.2)	0.01/0.02*
HWE	χ^2 = 0.02, P = 0.8	χ^2 = 0.16, P = 0.6		

OR = odds ratio; 95%CI = 95% confidence interval.
* Significant P/Pc values if ≤0.05, Pc = Bonforroni corrected P value (Number of comparison × P value).

Table 4
Distribution of *ApoE* alleles and genotypes in CHD patients and controls.

ApoE gene Polymorphism	CHD Patient (N = 100) N/%	Controls (N = 100) N/%	OR (95% CI)	P/Pc
Alleles (n = 200)				
E2	9/4.5	13/6.5	1	
E3	159/79.5	173/86.5	1.28 (0.49–3.4)	0.58/NS
E4	32/16	14/7	3.3 (1.02–10.94)	0.02/0.04*
Genotypes				
E2E3	9/9	13/13	1	0.7/NS
E3E3	59/59	73/73	1.17 (0.43–3.2)	
E3E4	32/32	14 /14	3.3 (1.02–10.9)	0.02/0.04*
HWE	χ^2=6.6, P = 0.009	χ^2=2.4, P = 0.11		

OR = odds ratio; 95%CI = 95% confidence interval.
* Significant P/Pc values if ≤0.05, Pc = Bonforroni corrected P value (Number of comparison × P value).

Table 5
Frequency of combined genotype of eNOS and ApoE in CHD patients and controls.

*eNOS/ApoE*gene Polymorphism	Patients (N = 100) (%)	Controls (N = 100) (%)	OR (95% CI)	P/Pc
GGE2E3	3	6	0.48 (0.09–2.3)	0.4/NS
GGE3E3	27	46	0.43(0.23–0.82)	0.005 /0.045
GGE3E4	10	8	1.3 (0.4–3.7)	0.6/NS
GTE2E3	3	7	0.4 (0.08–1.8)	0.2/NS
GTE3E3	26	24	1.1 (0.5–2.2)	0.7/NS
GTE3E4	17	3	6.6 (1.7–29.5)	≤0.001/0.009*
TTE2E3	3	0	Undefined	Not applicable
TTE3E3	6	3	2.06 (0.4–10.7)	0.4/NS
TTE3E4	5	3	1.7 (0.3–9.2)	0.7/NS

OR = odds ratio; 95%CI = 95% confidence interval.

differentiation was found with Fallah et al.[35], and Kambouh et al.[36] (from Iran and Nigeria respectively) (F_{ST} = 0.093, 0.066; respectively), while little genetic differentiation with ApoE gene were found with all other studies in Table 9. Al-Bustan et al.[45], and Cattin et al.[46] studies were excluded from comparison as they were not in HW equilibrium.

The differentiation with other Egyptian studies could be due to different sample sizes and due to different inclusion criteria as our study was done among 1st cardiac attack patients.

NB: The fixation index (F_{ST}) is a measure of population differentiation due to genetic structure. It is frequently estimated from genetic polymorphism data, such as single-nucleotide polymorphisms (SNP) or microsatellites. Our Statistician Used FSTAT a computer program to estimate and test gene diversities and statistics.

4. Discussion

The present study was conducted at Mansoura University Hospitals where most of admitted patients belonged to middle or lower socio-economic class; therefore, it was found that there was no statistically significant difference between both groups as regard to socio-demographic features that included (residence, education, occupation and income). The results in the present study are consistent with the previous observations of Xu et al.[47] in China and Panwar et al.[48] in India. On the contrary, Loock et al.[49] in South Africa found that most CHD cases had low socioeconomic background and limited education.

A number of linkages and candidate gene studies have been performed in the past decades to identify the genes characteristic of CHD. The Glu298Asp polymorphism of the human endothelial nitric oxide synthase gene is thought to be one of the genes associated with CHD. In the current study, a statistically significant association was observed between GT and TT genotypes of endothelial nitric oxide synthase gene with CHD with OR = 2.03 and 3.5; respectively). This also came in agreement with case control studies done by Motawi et al.[13] in Egypt and Luo et al.[50] in China who observed a statistically significant association between genotypes of endothelial nitric oxide synthase gene and the occurrence of CHD with ORs = 3.3, and 1.4; respectively.

Table 6
Relation between *eNOS* genotype polymorphism and baseline characteristics of CHD patients.

	eNOS genotype			P value
	GG (40)	GT (46)	TT (14)	
Chest pain				
Retrosternal (91)/Epigastric (9)	35(38.5)/5 (55.6)	43 (47.3)/3 (33.3)	13(14.3)/1(11.1)	P = 0.5*
Radiating pain: No (24)	15 (62.5)	8 (33.3)	1 (4.2)	
/Yes (76)	25 (32.9)	38 (50)	13 (17.1)	P = 0.8*
Family history of CHD:	27(45)	23 (38.3)	10 (16.7)	
No (60)/Yes (40)	13(32.5	23(57.5	4 (10)	P = 0.07*
Nature of pain Stabbing (52)	25 (48.1)	22 (42.3)	5 (9.6)	
Burning (25)	8 (32)	13 (52)	4 (16.)	
compressing (23)	7 (30.4)	11 (47.8)	5 (21.7)	P = 0.4*
ECG findings (N/%)				
STEMI (68)	28 (41.2)	30 (44.1)	10 (14.7)	
NONSTEMI(T wave inversion) (22)	7 (31.8)	11 (50)	4 (18.2)	
NONSTEMI (ST depression) (10)	5 (50)	5 (50)	0 (0)	P = 0.4*
ECHO finding:				
Segmental wall motion: (N/%)				
Anterior wall MI (45)	16 (35.6)	22 (48.9)	7 (15.6)	
Inferior wall (29)	13 (44.8)	12 (41.4)	4 (13.8)	
Lateral wall (26)	11 (42.3)	12 (46.2)	3 (11.5)	P = 0.6*
Lipid profile: (N)				
Cholesterol (mg/dL): <200 (19)/≥200 (81)	5 (26.3)/35 (43.2)	13 (68.4)/33 (40.7)	1 (5.3)/13 (16)	P = 0.07*
TG (mg/dL): <150 (33)/≥150 (67)	12 (36.4)/28(41.8)	14 (42.4)/32 (47.8)	7 (21.2)/7 (10.4)	P = 0.3*
LDL(mg/dL) : <130 (28)/≥130 (72)	7 (25)/33 (45.8)	18 (64.3)/28(38.9)	3 (10.7)/11 (15.3	P = 0.06*
HDL(mg/dL): >45 (65)/≤45 (35)	28 (43.1)/12(34.3)	31(47.7)/15 (42.9)	6 (9.2) /8 (22.9)	P = 0.16*

* Monte Carlo Significance test.

Table 7
Relation between *ApoE* genotype polymorphism and baseline characteristics of CHD patients.

	ApoE genotype			P value
	E2E3 N = 9	E3E3 N = 59	E3E4 N = 32	
Chest pain				
Retrosternal (91)	9 (9.9)	55 (60.4)	27 (29.7)	
Epigastric (9)	0 (0)	4 (44.4)	5 (55.6)	P = 0.2*
Radiating pain: No (24)	2 (8.3%)	17(70.8)	5 (20.8)	
Yes (76)	7 (9.2)	42 (55.3)	27 (35.5)	P = 0.4*
Family history of CHD: No (60)	4 (6.7)	38 (63.3)	18 (30)	
Yes (40)	5(12.5)	21 (52.5)	14 (3 5)	P = 0.4*
Nature of pain: Stabbing (52)	3 (5.8)	30 (57.7)	19 (36.5)	
Burning (25)	2 (8)	13 (52)	10 (40)	
Compressing(23)	4 (17.4)	16 (69.6)	3 (13)	P = 0.16*
ECG findings; (N/%)				
STEMI (68)	7 (10.3)	39 (57.4)	22 (32.3)	
NONSTEMI(T wave inversion) (22)	2 (9.1)	14 (63.6)	6 (27.3)	
NONSTEMI (ST depression) (10)	0 (0)	6 (60)	4 (40)	P = 0.5*
ECHO finding:				
Segmental wall motion: (N/%)				
Anterior wall MI (45)	5 (11.1)	29 (64.4)	11 (24.4)	
Inferior wall (29)	1 (3.4)	13 (44.8)	15 (51.7)	
Lateral wall (26)	3 (11.5)	17 (65.4)	6 (23.1)	P = 0.1*
Lipid profile: (N)				
Cholesterol (mg/dL): <200(19)/≥200 (81)	2(10.5)/7(8.6)	9 (47.4)/50 (61.7)	8 (42.1)/24 (29.7)	P = 0.5*
TG (mg/dL): <150 (33)/≥150 (67)	4 (12.1)/5 (7.4)	14 (42.4)/45 (67.2)	15 (45.5)/17 (25.4)	P = 0.06*
LDL(mg/dL): <130 (28)/≥130 (72)	3 (10.7)/6(8.4)	16 (57.1)/43 (59.7)	9 (32.2)/23 (31.9)	P = 1*
HDL(mg/dL): >45 (65)/≤45 (35)	7(10.8)/2 (5.7)	42 (64.6)/17 (48.6)	16 (24.6)/16(45.7)	P = 0.08*

* Monte Carlo Significance test.

It also appeared that the presence of the mutant T allele increased the risk of CHD 1.9 fold (95% CI = 1.2–3.1) and such finding coincides with that obtained by Angeline et al.[51] in India, Salimi et al.[11] in Iran where OR = 1.6 in both studies. On the other hand, no significant differences in the eNOS genotype or allele distribution pattern between the control subjects and the CHD patients as reported by Gad et al.[12], and Younan et al.[6] in Egypt, Afrasyap and Ozturk[52] in Turkey, Nassar et al.[53] in Canada.

These conflicting findings may be due in part to differences in the number and populations studied and different methods of case ascertainment. These findings further support the previously reported role of ethnicity in determining the prevalence of genetic polymorphisms and their subsequent putative impacts in a given population.

Concerning the Apo E gene polymorphism in the present study, it was observed that the carriers of E4 allele and especially E3/E4

Table 8
Comparison of genetic variability in studied eNOS SNPs between Egyptian healthy controls with other published studies.

Author	Publication year	Country	G	T	Fst P value	Reference
Sherihan Adel	2017	Egypt	0.770	0.230		Current
Gad	2012	Egypt	0.752	0.248	.002	12
Motawi et al.	2011	Egypt	0.660	0.340	.015	13
Diakite et al.	2014	Morocco	0.802	0.198	.002	14
Kerkeni	2006	Tunisia	0.779	0.221	<0.001	15
Alkharfy	2010	Saudi Arabia	0.814	0.186	.003	16
Yalcin et al.	2014	Turkey	0.784	0.216	<0.001	17
Bor-Kucukatay	2010	Turkey	0.818	0.182	.003	18
Alp	2009	Turkey	0.746	0.254	.001	19
Salimi et al.	2010	Iran	0.774	0.226	<0.001	11
Rahimi	2010	Iran	0.859	0.141	.013	20
Rai	2012	India	0.828	0.172	.005	21
Saini	2011	India	0.660	0.340	.015	22
Lin et al.	2008	Taiwan	0.776	0.224	<0.001	23
Ji	2007	China	0.911	0.089	.037	24
Wang	2007	China	0.663	0.337	.014	25
Vasilakou	2008	Greece	0.702	0.298	.006	26
Colombo et al.	2003	Italy	0.687	0.313	.009	27
Yoshimura	1998	Japan	0.955	0.045	.072	28
Shimasaki	1998	Japan	1.096	0.068	.187	29
da Costa Escobar Piccoli	2012	Brazil	0.743	0.257	.001	30
Isordia-Salas	2010	Mexico	0.861	0.139	.014	31
Zakrzewski-Jakubiak	2008	Canada	0.617	0.383	.027	32

Comparisons were done using pair wise fixation index (FST) comparison versus the current study.

Table 9
Comparison of genetic variability in studied ApoE SNPs between Egyptian healthy controls with other published studies.

Author	Publication year	Country	E2	E3	E4	Fst P value	Reference
Arafa et al.	2018	Egypt	0.065	0.865	0.070		Current
Halim et al.	2012	Egypt	0.067	0.917	0.017	0.005	33
Marrzoq et al.	2011	Gaza	0.082	0.815	0.103	0.003	8
Dzimiri et al.	1999	Saudi Arabia	0.050	0.888	0.063	0.001	34
Fallah et al.	2011	Iran	0.138	0.545	0.318	0.093	35
Kamboh et al.	1989	Nigeria	0.028	0.662	0.310	0.066	36
Balcerzyk	2007	Poland	0.051	0.879	0.070	<0.001	37
Kolovou	2005	Greece	0.058	0.806	0.136	.007	38
Peng	2001	China	0.082	0.828	0.090	.002	39
Batalla	2000	Japan	0.048	0.870	0.083	.001	40
Luc et al.	1994	France	0.081	0.802	0.117	0.006	41
van Bockxmeer	1992	Australia	0.061	0.811	0.128	.006	42
Hanis et al.	1991	Mexican Americans	0.039	0.859	0.102	0.002	43

Comparisons were done using pair wise fixation index (FST) comparison versus the current study.

genotype were at higher risk of CHD with OR = 3.3 (95% CI = 1.02–10.94) for both. These findings are more or less similar to that detected by Elmadbouh et al.[54] in Egypt, Attila et al.[55] in Turkey, Kharrazi et al.[56] in Iran who showed that the E3/E4 genotype was statistically significantly higher in CHD patients compared to the controls.

In contrast, others found that the association between E4 allele and CHD was negative such as studies done by Hsieh et al.[57] in Taiwan and Kolovou et al.[58] in Greece. This discrepancy regarding results might be explained by gene environment interactions in different ethnic populations and due to different sample size.

It was suggested that, although the risk associated with any single genotype is modest, in combination, they may be associated with a clinically high significant risk (Humphries et al.[59] According to the present study, combined genotypes was studied and significant association was observed between the presence of GTE3E4 genotype and CHD with OR = 6.6 (1.7–29.5). These findings are supported by a meta-analysis of Boekholdt et al.[60] who stated that the impact of APOE singly would not necessarily improve prediction, however, and the combination of SNPs in the genes for uncoupling protein 2 (UCP2), apolipoprotein AIV (APOA4), and lipoprotein lipase (LPL) with APOE improved prediction further.

In conclusion, it was shown in this study that Endothelial Nitric Oxide Synthase Glu 298 Asp (G894T) and Apolipoprotein *E* gene polymorphisms may contribute to the individual susceptibility of CHD. Further rigorous design, wide scale and multicentre studies, large sample of case-control, or prospective study are warranted to continue in-depth evaluation and investigation of the relationship between gene polymorphisms -either alone or combined- and the occurrence of CHD among Egyptian population.

5. Study limitations

This study is a group matched case control study design and the results are limited to the subgroup of survivors of CHD but not to the entire group of patients with CHD, these observations need further confirmation using prospective study design. Also, the sample size was not large enough due to high cost of genotyping. Another limitation was that the single center hospital based study that doesn't reflect the national situation at the community level.

6. Declaration of conflicting interests

The author(s) declared no conflicts of interest with respect to the authorship and/or publication of this article.

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