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

Sherif Arafa, Sherehan Abdelsalam, Abdel-Hady El-Gilany, Youssef Mohamed Mosaad, Adel Abdel-Ghaffar

Cardiovascular Medicine, Cardiovascular Department, Mansoura Faculty of Medicine, Mansoura, Egypt
Public Health and Preventive Medicine, Public Health & Community Medicine Department, Mansoura Faculty of Medicine, Mansoura, Egypt
Clinical Immunology Unit, Clinical Pathology Department & Mansoura Research Center for Cord Stem Cells (MARC_CSC), Faculty of Medicine, Mansoura University, Mansoura, Egypt

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

References:
1. https://doi.org/10.1016/j.ehj.2018.08.001
2. 1110-2608/© 2018 Egyptian Society of Cardiology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Endothelial NO is synthesized by the enzyme eNOS that is encoded by the gene located on chromosome 7q35-3q6. 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 ApoE4 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 AAC GAG CTG CAG GCG GCG-3′; reverse primer 5′-GAC CCT GGA GAT GAA GGC AGG AGA-3′; eNOS; forward primer 5′-GAC CCT GGA GAT GAG GAG-3′ and reverse primer 5′-ACC TCC AGG ATG TTG TAG CGG-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 AfillI 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.
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...
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 signif-
icant (P = 0.02), with Bonforroni correction (Pc = 0.04). The pres-
ence 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 con-
trol 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 < 0.001) as the pres-
ence 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 polymor-
phisms 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 coun-
tries using pair wise fixation index (FST). Moderate genetic differ-
etiation 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 inTable 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.

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                | χ² = 0.03        | 1.07 (0.5–2.3) |
| 60+ ys                           | 36             | 36                | χ² = 0.01        | 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                |                  | Undefined |
| Male                             | 85             | 85                |                  |           |
| Residence                        |                |                   |                  |           |
| Rural(r)                         | 39             | 38                |                  | 1         |
| Urban                            | 61             | 62                | χ² = 0.02        | 0.96 (0.02–1.7) |
| Education                        |                |                   |                  |           |
| Illiterate/Read and write(r)     | 41             | 39                |                  | 1         |
| Secondary or less                | 48             | 46                | χ²              | 0.99 (0.52–1.8) |
| More than secondary              | 11             | 15                | χ²              | 0.7 (0.26–1.8) |
| Occupation                       |                |                   |                  |           |
| Non-working/housewife(r)         | 12             | 6                 |                  | 1         |
| Manual /Farmer/Trades            | 51             | 51                | χ²              | 0.5 (0.15–1.5/0.43 (0.13–1.4) |
| Semi prof/Professional           | 37             | 43                | χ²              |           |
| Income                           |                |                   |                  |           |
| Sufficient (r)                   | 11             | 19                |                  | 1         |
| Insufficient                     | 89             | 81                | χ²              | 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               | 24/76         |
| Radiating pain: No/Yes          |                    |               |
| Site of radiation (N/%)         |                    |               |
| Left shoulder                   | 59 (77.6)          | 61 (75.9)     |
| Right shoulder                  | 5 (6.6)            | 8 (9.6)       |
| Left arm                        | 25 (32.9)          | 30 (37.7)     |
| Right arm                       | 4 (5.3)            | 4 (4.8)       |
| Back                            | 46 (60.5)          | 43 (53.7)     |
| Nature of pain                  |                    |               |
| Stabbing/Burning/Compressing    | 52/25/23           | 51/25/23      |
| Precipitating factors: (N/%)    |                    |               |
| Stress                          | 3 (3)              | 3 (3)         |
| Heavy work                      | 22 (22)            | 22 (22)       |
| Heavy meal                      | 12 (12)            | 12 (12)       |
| During quite sleep              | 58 (58)            | 58 (58)       |
| During setting                  | 5 (5)              | 5 (5)         |
| Duration of pain (Mean ± SD)    | 3.4 ± 1.165        | 3.4 ± 1.165   |
| ECG findings; (N/%)             |                    |               |
| STEMI                           | 68 (68)            | 68 (68)       |
| NONSTEMI/T wave inversion       | 22 (22)            | 22 (22)       |
| NONSTEMI (ST depression)        | 10 (10)            | 10 (10)       |
| ECHO finding                    |                    |               |
| Ejection fraction(Mean ± SD)    | 54.7 ± 4.7         | 54.7 ± 4.7    |
| Segmental wall motion: (N/%)    |                    |               |
| Anterior wall MI                | 45 (45)            | 45 (45)       |
| Inferior wall                   | 29 (29)            | 29 (29)       |
| Lateral wall                    | 26 (26)            | 26 (26)       |
| Lipid profile: (N)              |                    |               |
| Cholesterol (mg/dL); <200/200   | 19 / 81            | 17 / 81       |
| TG (mg/dL); <150/≥150           | 31 / 67            | 31 / 67       |
| LDL(mg/dL): <130/≥130           | 28/72              | 28/72         |
| HDL(mg/dL); >45/≤45             | 65/35              | 65/35         |

* Categories are not mutually exclusive. (Percent calculated from patients with radiating pain).
NB: The fixation index (FST) 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 3

| eNOS Polymorphism | CHD Patient (N = 100) | Controls (N = 100) | 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                | $\chi^2 = 0.02, P = 0.8$ | $\chi^2 = 0.16, P = 0.6$ |             |      |

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

Table 4

| ApoE gene Polymorphism | CHD Patient (N = 100) | Controls (N = 100) | 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                   | 99/59                 | 73/73              | 1.17 (0.43–3.2) | 0.7/NS |
| E3E4                   | 32/32                 | 14/14              | 3.3 (1.02–10.9) | 0.02/0.04* |
| HWE                    | $\chi^2=6.6, P=0.009$ | $\chi^2=2.4, P=0.11$ |             |      |

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

Table 5

| 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 |
| GTGE2E3                     | 3                      | 7                      | 0.4 (0.08–1.8) | 0.2/NS |
| GTGE3E3                     | 26                     | 24                     | 1.1 (0.5–2.2) | 0.7/NS |
| GTGE3E4                     | 17                     | 3                      | 6.6 (1.7–29.5) | 0.003/0.009* |
| 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.
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 Ozturk52 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
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.

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.

---

**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| .002 |         | Current   |
| Gad               | 2012             | Egypt     | 0.752| 0.248| .015 |         | 12        |
| Motawwi 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| .001 |         | 15        |
| Alharfy           | 2010             | Saudi Arabia | 0.814| 0.186| .003 |         | 16        |
| Yalcın et al.     | 2014             | Turkey    | 0.784| 0.216| <0.001|         | 17        |
| Bor-Cucukatay     | 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|         | 20        |
| Rahimi            | 2010             | Iran      | 0.859| 0.141| .013 |         | 21        |
| Saini             | 2012             | India     | 0.828| 0.172| .005 |         | 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| .005 |         | Current   |
| Halim et al.      | 2012             | Egypt     | 0.067| 0.917| 0.017| .005 |         | 33        |
| Marrzqo et al.    | 2011             | Gaza      | 0.082| 0.815| 0.103| .003 |         | 8         |
| Dizmiri et al.    | 1999             | Saudi Arabia | 0.050| 0.888| 0.063| .001 |         | 34        |
| Fallah et al.     | 2011             | Iran      | 0.138| 0.545| 0.318| .093 |         | 35        |
| Kamboh et al.     | 1980             | Nigeria   | 0.028| 0.662| 0.310| .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        |
| Batalia           | 2000             | Japan     | 0.048| 0.870| 0.083| .001 |         | 40        |
| Luc et al.        | 1994             | France    | 0.081| 0.802| 0.117| .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| .002 |         | 43        |

Comparisons were done using pair wise fixation index (FST) comparison versus the current study.
58. Kolovou GD, Anagnostopoulou KK, Cokkinos DV. Apolipoprotein E gene polymorphism and myocardial infarction. *Int J Cardiol*. 2009;133:264–265.

59. Humphries SE, Cooper JA, Talmud PJ, Miller GJ. Candidate gene genotypes, along with conventional risk factor assessment, improve estimation of coronary heart disease risk in healthy UK men. *Clin Chem*. 2006;53:8–16.

60. Boekholdt SM. Cholesteryl ester transfer protein TaqIB variant, high-density lipoprotein cholesterol levels, cardiovascular risk, and efficacy of pravastatin treatment: individual patient meta-analysis of 13,677 subjects. *Circulation*. 2005;111:278–287.