Research article

Apoprotein-E gene polymorphism in an Iraqi population with type 2 diabetes and cardiovascular disease

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ABSTRACT

Introduction and Aim: The ApoE gene polymorphisms are considered as risk factors for developing atherosclerosis and related cardiovascular disease (CVD) in humans. There exists no study pertaining to ApoE gene polymorphism and its association to these disorders among the Iraqi population. Hence in this study, we aimed to investigate the possible relationship between single nucleotide polymorphisms (SNPs) in the ApoE gene with the prevalence of diabetes (T2DM) and cardiovascular disease among the Iraqi population.

Materials and Methods: This cross-sectional investigation involved 76 patients (50 with diabetics and 26 with cardiovascular disease) and 73 otherwise healthy individuals. The ApoE gene fragment corresponding to the SNPs rs429358 and rs7412 was amplified using conventional polymerase chain reaction (PCR) with a specific pair of primers. Genotyping was performed by direct sequencing.

Results: Differences in genotypic and allelic frequencies were seen in SNPs rs429358 and rs7412 of the ApoE gene. Significant higher frequency was seen for the CT genotype in SNP rs429358 when compared to healthy controls. Among the epsilon alleles, the ε3/ε3 was the most common genotype, accounting for 76% and 69.23% of patients with diabetes and CVD, respectively. ε2/ε4 was the least common genotype, accounting for 2% and 0% of diabetes and CVD patients, respectively. When DM patients were compared to controls, the genotype ε 3 ε 4 was found to be more common in T2DM patients (10%) than in controls (1.34%), with a significant difference (OR= 4.32, 95%CI=0.02-0.98, p= 0.050). At the allelic level, the ε2 allele was significantly more common in patients than in controls (OR= 12.73, 95%CI= 1.38-116.87).

Conclusion: Data in this study indicate ApoE gene polymorphisms in SNPs rs429358 and rs7412 could be risk factors for T2DM and CVD in Iraqi patients.

Keywords: Apoprotein E; type 2 diabetes mellitus; cardiovascular disease; gene polymorphism.

INTRODUCTION

Hyperlipidemia is considered an important risk factor for atherosclerosis and related cardiovascular disease (CVD) (1) which are affected by several factors including environmental, lifestyle, cholesterol, and genetic factors (2). The ApoE gene polymorphism is considered one of the risk factors for these disorders (3,4).

ApoE gene is located on chromosome 19 at position q13.2a. The ApoE glycoprotein consists of 299 amino acids, and has three alleles *ε2*, *ε3*, and *ε4,* differing from one another by one amino acid substitution (5,6). The glycoprotein is known to regulate physiological activities such as lipid metabolism, cognitive function, and immune regulation (5). The ApoE alleles *ε2*, and *ε4* together with other allelic genes are considered risk factors in dysbeta-lipoproteinemia (DBL) susceptibility which is associated with both T2DM and CVD (5,6).

In 2007, Bennet *et al*., (7) demonstrated a linear relationship between ApoE genotypes, LDL-C levels and the risk of coronary disease. Different ApoE gene alleles have been linked to different types of hyperlipidaemia. Higher triglycerides were seen with *ε2* alleles, and higher cholesterol and LDL-C with *ε4* alleles (8). ApoE gene polymorphisms have been shown to play a role in the efficacy of statins used in the treatment of hyperlipidemia (4).

ApoE3 is responsible for the conversion of very low-density lipoprotein (VLDL) and its remnants to LDL in the liver, whereas ApoE2 has been shown to inhibit this process, resulting in the appearance of high cholesterol-containing remnants in the blood, which can lead to premature coronary heart disease (5). Homozygous carriers of ApoE2 (Arg158→Cys158) are reported to develop hyperlipidemia (6), while few reports showed a strong association to the *ε4* allele with the risk of CVD (9), and a cause of increased risk especially in diabetic patients (10).

The prevalence of ApoE gene alleles was found to be 0.061, 0.904, and 0.035 in normal Iraqi Kurdish people for APOE2, APOE3, and APOE4, respectively (11). However, there are no reports of ApoE gene polymorphism, or the prevalence of diabetes and cardiovascular disease among the Iraqi population. Hence, the present study aimed to investigate the association of ApoE gene polymorphisms (rs 429358 and rs7412) with T2DM and CVD among Iraqi patients.

MATERIALS AND METHODS

Study subjects and samples

This is a cross-sectional study that included 50 patients with T2DM and 26 patients with CVD, who were attending Al-Imamain Al-Kadhumain Medical City, Baghdad during the period from October 2010 to February 2021. The inclusion criteria for T2DM patients were fasting blood sugar ≥126 mg/dl, while for CVD patients, echocardiography was the principal tool. Family unrelated, age- matched apparently healthy 73 individuals were selected to represent the control group. Once written informed consent was provided, the subject's demographic information was obtained.
About 2 ml of venous blood was collected from each subject in tubes containing EDTA and proceeded further for DNA extraction, amplification, and genotyping.

DNA extraction, Gene amplification and genotyping

DNA was extracted from whole blood using a ready commercial kit (gSYNCTM DNA Mini Kit Whole Blood Protocol/ Genaid/ Taiwan) following manufacturer's instructions. ApoE gene fragments (335 bp) corresponding to SNP rs429358 and SNP rs7412 of the gene were amplified using specific primer pairs (Bioneer, Korea). The primer sequences used were as follows: Fwd: 5'-TGTAAAACGACGGCCATGGGCACGGCTGTCC AA-3` and Rev: 5'-CAGGAAACAGCTATGACCG CGGCCCTGTTCCA-3`. PCR reaction was carried out in a total volume of 25µl, containing 50 ng of genomic DNA, 1.5 µl of 10×PCR buffer, 0.3 µl of 10 mM dNTPs, 0.25 µl of 10 pmol/µl of each primer, and 1.25 U of Taq DNA polymerase (Bioneer/Korea). The amplification was carried out in a thermocycler ABI 9600 (Hybaid, England) with cycling conditions set up as follows: 94°C for 2 min; 35 cycles at 94°C for 30 s, 60°C for 45s, and 72C for 30 s; and a final extension step at 72°C for 5 min.

The amplified PCR products were excised, and outsourced for sequencing (Macrogen, Korea). The resultant sequences obtained were subjected to comparative sequence analysis using Basic Local Alignment Search Tool (BLAST) available at https://blast.ncbi.nlm.nih.gov/Blast.cgi.

Statistical analysis

The SPSS program version 25.0 was used for statistical analysis (SPSS, Chicago). The mean and standard deviation of continuous data were calculated and examined using analysis of variance (ANOVA). Categorical variables were reported as numbers and percentages, and the Chi-square test was used to examine them. In order to analyze the association between the polymorphism and MD and CVD risk, binary logistic regression was used to generate the odds ratio (OR) and the related 95% confidence intervals (CI). A p-value of 0.05 was regarded as significant.

RESULTS

Demographic characteristics of the study population

The mean age of the patients with T2DM and CVD was similar to that of controls with no significant difference. Females were more frequent in the T2DM group (60%) than either CVD group (53.85%) or controls (50.9%) with no significant differences. The mean BMI in controls was 28.11±5.98 kg/m^2 which was significantly lower than patients with T2DM and CVD (31.74±6.37 kg/m^2 and 32.1±2.83kg/m^2, respectively) as shown in Table 1.

Table 1: Demographic characteristics of the study population

Variables	Diabetes (n=50)	CVD (n=26)	Controls (n=73)	p- value
Age, years Mean±SD Range	 46.48±9.3 25-67	 43.15±7.34 29-57	 43.51±11.47 18-56	 0.268
Gender Male Female	 20(40%) 30(60%)	 12(46.15%) 14(53.85%)	 30(41.1%) 43(50.9%)	 0.868
BMI Mean±SD Range	 31.74±6.37[a] 21.33-49.05	 32.1±2.83[a] 20.31-41.67	 28.11±5.98[b] 18.69-46.88	 **0.003**

Different lower-case letters indicate significant differences

Association of Apo E gene polymorphisms with DM and CVD

Two SNPs in ApoE gene were investigated in their association with DM and CVD using conventional PCR with specific sets of primers. Fig. 1 shows the gel electrophoresis for the ApoE gene fragment corresponding for the two SNPs: rs429358 and rs7412. Genotyping was performed through direct sequencing.

Sequencing of SNP rs429358 showed the TT, CT and TT genotypes (Fig.2A), while the SNP rs7412 showed the CC and CT genotypes (Fig: 2B). The genotypic and allelic frequencies for TT and CT genotypes within SNP rs429358 among patients suffering from diabetes and cardiovascular disease (CVD) is presented in Table 2. As seen from Table, no significant difference was seen for the presence of genotypes between the two disorders. Although allele C was more frequent in CVD patients (7.69%) than T2DM patients (6%) the difference was not significant (Table 2).

A comparison of SNP rs429358 genotypes between T2DM patients and controls, showed the frequency of the CT genotype to be significantly higher in patients (p=0.037; OR= 9.82, 95% CI= 1.14-84.28) compared to healthy individuals (Table 2). Similarly, significant difference (p=0.025), was also observed for the CT genotype between CVD patients and the control group. As shown in Table 2, the C allele was more common in T2DM than controls (6% vs 0.7%), with a significant difference (OR=9.25, 95% CI=1.1-78.1). There was no significant difference in the genotype distribution between patients with T2DM and CVD (Table 2).

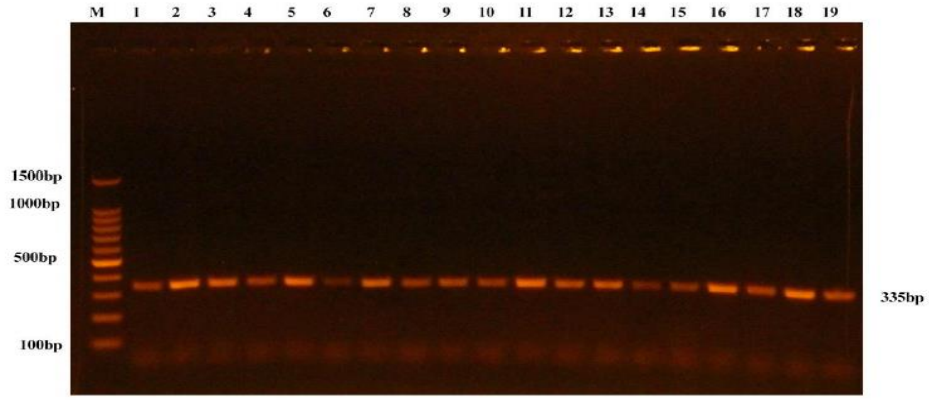

Fig.1: Gel electrophoresis of ApoE gene fragment (335 bp) corresponding to SNP rs429358 and rs7412.

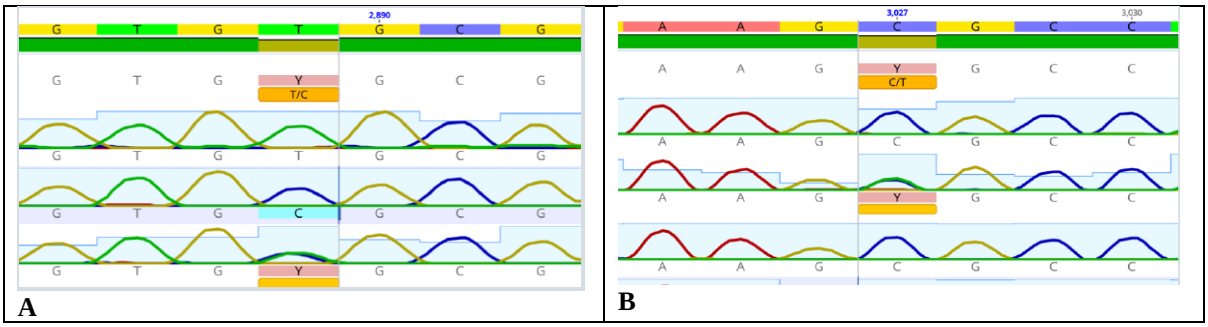

Fig. 2: Chromatogram of **A**. SNP rs429358 and **B**. SNP rs7412

Table 2: The genotype and allelic frequency in SNPrs429358 for diabetic and CVD patients

SNP rs429358	Diabetes (DM) (n=50)	CVD (n=26)	Controls (n=73)	*P*-values			OR (95%CI)		
				DM vs control	CVD vs control	DM vs CVD	DM vs control	CVD vs control	DM Vs CVD
Genotypes									
TT	44(88%)	21(84.62%)	72(98.63%)	**0.037**	**0.025**	0.697	1.0	1.0	1.0
CT	6(12%)	4(15.38%)	1(1.36%)				9.82(1.14-84.28)	13.09 (1.39-123.3)	0.75 (0.19- 2.93)
CC	0(0%)	1(3.85%)	0(0%)						
HWE	0.652	0.671	0.953						
Alleles									
T	94(4%)	46(88.46%)	145(99.3%)	**0.041**	**0.027**	0.690	1.0	1.0	1.0
C	6(6%)	6(11.54%)	1(0.7%)				9.25 (1.1-78.1)	12.08 (1.32-110.75)	0.77 (0.21-2.84)

HWE: Hardy-Weinberg equilibrium, Bold numbers : Significant p-values

Table 3: The genotype and allelic frequency in SNPrs 7412 for diabetic and CVD patients

SNP rs7412	Diabetes (DM) (n=50)	CVD (n=26)	Controls (n=73)	*P*-values			OR (95%CI)		
				DM vs control	CVD vs control	DM vs CVD	DM vs control	CVD vs control	DM vs CVD
Genotypes									
TT	43(86%)	22(84.62%)	68(93.15%)	0.198	0.205	0.871	1.0	1.0	1.0
CT	7 (14%)	4 (15.38%)	5(6.85%)				2.21(0.66-7.42)	2.47 (0.61-10.03)	0.89 (0.24-3.39)
HWE	0.594	0.671	0.762						
Alleles									
T	93 (93%)	48(92.1%)	141(96.58%)	0.21	0.216	0.876	1.0	1.0	1.0
C	7 (7%)	4(7.69%)	5(3.42%)				2.12(0.65-6.89)	2.35(1.61-9.11)	0.9 (0.25-3.24)

In the case of SNP rs7412, the frequency of the CT genotype was slightly higher in T2DM and CVD patients when compared to healthy controls, with the difference however, seen to be not significant (Table 3). At allelic level, the C allele was more frequent in T2DM than controls (7.69% vs 3.42%) with no significant difference. Similarly, there was no significant differences between CVD patients and controls neither in genotype distribution or allele frequency (Table 3).

Genotyping according to different epsilon alleles

Different epsilon genotypes are formed for each allele in SNP rs429358 with alleles in SNP rs7412. Based on the results of this study, there were four epsilon genotypes E3E3, E2E3, E3E4 and E2E4. In general, the most common genotype was E3E3 representing 76% and 69.2% of patients with T2DM and CVD, respectively. The least common genotype was E2E4 accounting for 2% and 0% of patients with T2DM and CVD, respectively. As E3E3 genotype was the most common, it was considered as the wild genotype. On the other hand, allele E3 accounted for the vast majority of cases both in DM and CVD (87% and 84.62%, respectively). The distribution of genotypes and allele was comparable between T2DM and CVD patients with no significant difference (Table 4).

A comparison between T2DM patients with controls revealed that the genotype E3E4 was more common among DM patients than controls (1.34%) with a significant difference (p value=0.05 and OR= 4.32, 95%CI=0.02-0.98). Furthermore, the frequency of E4 allele in patients was 6% which was higher than that of controls (0.68%) showing significant difference (p= 0.037, and OR= 9.65, 95%C=1.14-81.56) as shown in Table 4.

A comparison between CVD patients and controls demonstrated the higher frequency of E2E3 and E3E4 genotypes (15.38% for each) in patients than controls (6.85% and 1.34%, respectively) with significant differences (OR= 15.0, 95%CI= 1.56-141, p= 0.019 and OR= 5.0,95%=1.38-64.37, p=0.016, respectively). At the allelic level, the E2 allele was significantly more common in CVD patients than in controls (p value= 0.025, OR= 12.73, 95% CI= 1.38-116.87) (Table 4).

DISCUSSION

Our study showed the C allele of rs429358 to be significantly associated with T2DM and CVD, while the T allele of rs7412 to be significantly associated with T2DM. These findings are consistent with previous studies (10). Furthermore, allele E4 (representing allele C of rs429358 and allele C of rs7412) was significantly linked to diabetes, while allele E2 (representing allele T of both SNPs) was linked to CVD. These findings support previous research that found these SNPs to be important in T2DM and CVD.

Table 4: The genotypic and allelelic frequency in epsilon (ε) alleles in diabetic and CVD patients

Epsilon alleles	Diabetes (DM) (n=50)	CVD (n=26)	Controls (n=73)	*P*-values			OR (95%CI)		
				DM vs control	CVD vs control	DM vs CVD	DM vs control	CVD vs control	DM vs CVD
Genotypes									
ε3/ε3	38(76%)	18 (69.23%)	67(91.78%)	0.169	**0.027**	0.838	1.0	1.0	1.0
ε2/ε3	6(12%)	4 (15.38%)	5(6.85%)	0.241	**0.019**	0.628	1.33(0.14-1.65)	**15.0(1.56-141)**	1.41 (0.35-5.62)
ε3/ε4	5(10%)	4 (15.38%)	1(1.34%)	**0.050**	**0.016**	0.472	**4.32(0.02-0.98)**	**5.0 (1.38-64.37)**	1.69 (0.4-7.05)
ε2/ε4	1(2%)	0 (0%)	0(0%)	0.692	**1.00**	0.605	1.4(0.43-25.4)	**1.0 (1.0-1.0)**	2.11 (0.12-35.7)
Alleles									
ε3	87(87%)	44(84.62%)	140(95.89%)	0.050	**0.036**	0.908	1.0	1.0	1.0
ε2	7(7%)	4(7.69%)	5(3.42%)	0.236	**0.025**	0.852	4.28(0.38-47.62)	12.73(1.38-116.87)	1.13(0.31-4.07)
ε4	6(6%)	4(7.69%)	1(0.68%)	**0.037**	0.217	0.681	9.65(1.14-81.56)	5.0(0.39-64.38)	1.32 (0.35-4.91)

Bold indicates significant values.

ApoE polymorphism and CAD have been linked in numerous ethnic groups, including Caucasian in the United States (12), Austrian (13) and Indian (14) populations. Other studies have found the ApoE 4 allele to be an independent risk factor for the development of CAD in T2DM (15) and myocardial infarction after adjusting for other known risk variables (14).

Clark *et al.,* (16) found that both the allele and genotype distribution of rs429358 were linked with diabetes (p = 0.04 and p = 0.05, respectively) in a British cohort. Zheng *et al.,* (17) examined the relationship between ApoE gene polymorphism and T2DM complicated by CAD in the Chinese population first. The findings revealed that the APOE-4 allele enhanced the risk of CAD in T2DM, which was similarly shown in previous similar investigations (18). However, the ApoE-2 allele was discovered to be related with the risk of CAD in T2DM patients (19). Nevertheless, several investigations revealed no relationship between ApoE 2/3/4 polymorphisms and the incidence of CAD in T2DM patients (20).

Song *et al*., (21) demonstrated that ApoE-ε4 allele carriers had a 42% increased risk for CAD in relative those carrying ε3/ε3 genotypes. Almost similar results were obtained by Xu *et al*., (22) who revealed that the ε4 allele had a 46% higher risk of CAD in the Chinese population (OR = 1.46, 95% CI = 1.28– 1.66). These results are in accordance with many other studies (23,24). In a meta-analysis, Wu *et al*., (25) disclosed that ApoE-ε4 allele is linked with increased risk of T2DM patients with CAD in the Chinese population. Interestingly, it was reported ethnicity is a crucial factor in determining the risk of ApoE-ε2 allele in CVD (22).

The three ApoE alleles (ε2, ε3, ε4) differ by only one or two amino acids. However, these insignificant differences can dramatically affect the structure and function of ApoE. In general, the ApoE-ε4 allele is linked with higher and the APOE-ε2 allele with lower serum cholesterol as well as low density lipoprotein compared with the APOE-ε3 allele (8). Accordingly, disturbance in lipoprotein metabolism may describe, at least in part, the connection between ApoE ε2/ε3/ε4 polymorphisms and the development of CAD in patients with T2DM. Thus, the results obtained in this study show that ApoE gene polymorphisms in SNPs rs429358 and rs7412, could be important risk factors for T2DM and CVD probably interfering with lipid parameters. Further studies regarding the effect of different genotypes on lipid profile in the Iraqi population are required in order to illustrate the mechanism(s) by which ApoE polymorphisms can predispose patients for T2DM or CVD.

CONCLUSION

Our findings suggest that ApoE gene polymorphisms in SNP rs429358 and SNP rs7412 could probably be considered important risk factors for T2DM and CVD among Iraqi the population.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest.

REFERENCES

1. Miao, J., Zang, X., Cui, X., Zhang, J. Autophagy, hyperlipidaemia, and atherosclerosis. Adv Exp Med Biol. 2020; 1207:237-264.
2. Meng, Q., Zhang, X. H., Zhang, X. W. Meta-analysis on association of ApoE gene polymorphism with hyperlipidaemia. Chinese Preventive Med. 2015; 16:304-307.
3. Zhao, X. N., Sun, Q., Cao, Y. Q. Association between apolipoprotein gene polymorphisms and hyperlipidaemia: a meta-analysis. BMC Genom Data 2021; 22, 14 doi.org/10.1186/s12863-021-00968-1
4. Cai, C., Wen, Z., Li, L. The relationship between Apo E gene polymorphism and the efficacy of statins controlling hyperlipidaemia. Am J Transl Res. 2021; 13(6): 6772–6777.
5. Boot, C. S., Luvai, A., Neely, R. D. G. The clinical and laboratory investigation of dysbetalipoproteinemia. Critical Reviews in Clinical Laboratory Sciences. 2020; 57(7):458-469.
6. Hameed, S. T., Al-Mayah, Q. S., Khudeir, M. S., Al-Shamma, G. A. Estimation of dysbetalipoproteinemia incidence in Iraqi patients with diabetes mellitus and cardiovascular disease. PJMHS 2022; 16:469-472.
7. Bennet, A. M., Di Angelantonio, E., Ye, Z., Wensley, F., Dahlin, A., Ahlbom, A., *et al.,* Association of apolipoprotein E genotypes with lipid levels and coronary risk. JAMA. 2007 Sep 19; 298(11):1300-1311.
8. Larifla, L., Armand, C., Bangou, J., Blanchet-Deverly, A., Numeric, P., Fonteau, C., *et al.,* Association of APOE gene polymorphism with lipid profile and coronary artery disease in Afro-Caribbeans. PLoS ONE 2007; 12:e0181620. doi:10.1371/journal.pone.0181620
9. Hou, J., Deng, Q., Guo, X., Deng, W., Zhong, W., Zhong, Z. Association between apolipoprotein E gene polymorphism and the risk of coronary artery disease in Hakka postmenopausal women in southern China. Lipids Health Dis, 2020; 19, 139, doi.org/10.1186/s12944-020-01323-6
10. Liu, S., Liu, J., Weng, R., Gu, X., Zhong, Z. Apolipoprotein E gene polymorphism and the risk of cardiovascular disease and type 2 diabetes. BMC Cardiovasc Disord. 2019; 19(1):213-219.
11. Al-Jaf, S. frequencies of Apolipoprotein E polymorphism in Iraqi Kurdish population. Biology Metagene 2021; 28: 6. doi.10.1016/J.MGENE.2021.100867.
12. Eichner, J. E., Kuller, L. H., Orchard, T. J., Grandits, G. A., McCallum, L. M., Ferrell, R. E., *et al.,* Relation of apolipoprotein E phenotype to myocardial infarction and mortality from coronary artery disease. Am J Cardiol 1993; 71:160-165.
13. van Bockxmeer, F. M., Mamotte, C. D. Apolipoprotein epsilon 4 homozygosity in young men with coronary heart disease. Lancet 1992; 340:879-880.
14. Kumar, P., Luthra, K., Dwivedi, M., Behl, V. K., Pandey, R. M., Misra, A. Apolipoprotein E gene polymorphisms in patients with premature myocardial infarction: a case-controlled study in Asian Indians in North India. Ann Clin Biochem 2003, 40:382-387.
15. Guang-da, X., You-ying, L., Zhi-song, C., Yu-sheng, H., Xiang-jiu, Y. Apolipoprotein e4 allele is a predictor of coronary artery disease death in elderly patients with type 2 diabetes mellitus. Atherosclerosis. 2004; 175:77-81.
16. Clark, D., Skrobot, O., Adebiyi, I., Susce, M., De Leon, J., Blakemore, A., Arranz, M. Apolipoprotein-E gene variants associated with cardiovascular risk factors in antipsychotic recipients. Eur. Psychiatry. 2009; 24(7): 456-463.

17. Zheng, Y. M., Sun, R., Li, X. Y., Zhu, D., Gao, M., Zhao, L. B., *et al.*, Relationship between ApoE gene polymorphism and type 2 diabetes mellitus with its cardiovascular complications in Chinese. Chin. J. Endocrinol. Metab. 1998;14: 11-14.
18. Chaaba, R., Attia, N., Hammami, S., Smaoui, M., Ben Hamda, K., Mahjoub, S., *et al.*, Association between apolipoprotein E polymorphism, lipids, and coronary artery disease in Tunisian type 2 diabetes. J. Clin. Lipidol. 2008; 2:360-364.
19. Halim, E. F., Reda, A. A., Hendi, A. A., Zaki, S. A., Essa, E. S., Khalifa, A. S. Apolipoprotein E gene variants as a risk factor for coronary artery disease in type 2 diabetic Egyptian patients. Egypt. J. Immunol. 2012; 19:1-10.
20. Guo, J. J., Ju J., Xu, X. H. Association of polymorphisms of apolipoprotein E gene and high-sensitive C-reactive protein with type 2 diabetes with coronary heart disease. Shaanxi Med. J. 2007; 36:1613-1616.
21. Song, Y., Stampfer, M. J., Liu S. Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. Ann. Intern. Med. 2004; 141:137-147.
22. Xu, M., Zhao, J., Zhang, Y., Ma, X., Dai, Q., Zhi, H., *et al.*, Apolipoprotein E gene variants and risk of coronary heart disease: a meta-analysis. Biomed Res. Int. 2016; 2016:3912175.
23. Zhang, M. D., Gu, W., Qiao, S. B., Zhu, E. J., Zhao, Q. M., Lv, S. Z. Apolipoprotein E gene polymorphism and risk for coronary heart disease in the Chinese population: a meta-analysis of 61 studies including 6634 cases and 6393 controls. PLoS ONE 2004; 9:e95463.
24. Zhang, Y., Tang, H. Q., Peng, W. J., Zhang, B. B., Liu, M. Meta analysis for the association of apolipoprotein E epsilon2/epsilon3/epsilon4 polymorphism with coronary heart disease. Chin. Med. J. 2015;128: 1391-1398.
25. Wu, Z. R., Chen, Z. C., Fu, M. X., Chen, J. Y., Han, L. Y., Zhou, L. H., *et al.*, Polymorphism of apolipoprotein E gene and type 2 diabetic patients with coronary heart disease among Chinese Han population: a meta-analysis. J. Clin. Exp. Med. 2015; 14:982-985.