http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2020.306.312

Research Article
Contribution of Apolipoprotein E Isoforms to Colorectal Cancer Carcinogenesis

[1]Manar F. Alharbi, [1,2,3]Ayat B. Al-Ghafari, [1]Huda A. Al Doghaither, [1]Huda F. Alshaibi and [1]Ulfat M. Omar

[1]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[2]Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia
[3]Cancer and Mutagenesis Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

Background and Objective: Colorectal cancer (CRC) is one of the most common cancers worldwide. In 2018, CRC was considered the 3rd deadliest cancer globally. Several risk factors are associated with CRC carcinogenesis such as lipid metabolism alterations. Apolipoprotein is a key regulator in lipid metabolism as it controls cholesterol and triglyceride levels and facilitates the clearance of cholesterol and low-density lipoprotein (LDL) cholesterol from plasma. In this study, (*apoE Hhal*) genotypes were investigated in Saudi CRC patients to evaluate the contribution of these genotypes to CRC carcinogenesis. **Materials and Methods:** This case-control study included 66 CRC patients and 65 controls. The gDNA samples were collected from blood and then different *apoE* genotypes were determined by PCR-RFLP assay. **Results:** The four genotypes were distributed in CRC patients (E2/E3, E3/E3, E3/E4 and E4/E4), whereas, in controls, only two genotypes were determined (E2/E3 and E4/E4). However, none of the genotypes of the *apoE* gene were correlated with CRC risk ($p>0.05$). Interestingly, the E2/E3 and E4/E4 genotypes were the most distributed *apoE* genotypes in high grade CRC patients. **Conclusion:** Data from the current study might shed light on the correlation of *apoE* genotypes with CRC progression. However, more studies are required to reveal the molecular mechanisms that affect lipid metabolism.

Key words: *apoE* polymorphism, *apoE Hhal* genotypes, colorectal cancer progression, apolipoprotein, PCR-RFLP

Citation: Manar F. Alharbi, Ayat B. Al-Ghafari, Huda A. Al Doghaither, Huda F. Alshaibi and Ulfat M. Omar, 2020. Contribution of apolipoprotein E isoforms to colorectal cancer carcinogenesis. Pak. J. Biol. Sci., 23: 306-312.

Corresponding Author: Ayat B. Al-Ghafari, Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
Tel: +999504686549

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer globally after lung and breast cancers. In 2018, in both sexes combined, CRC was considered the second deadliest cancer worldwide, after lung cancer, as 9.2% of all cancer cases were attributed to CRC. It is also considered the third leading cause of cancer-related death among males with (924 mortality cases) and the second among females with (429 mortality cases) recorded[1] in 2018. The incidence of CRC varies widely by the region[2]. In Saudi Arabia, according to the latest cancer incidence report from the Saudi Cancer Registry (SCR), CRC ranked as the most common cancer in males and the third among females[3].

Usually, CRC begins as an abnormal growth (called a polyp), these small growths are often benign but some have the ability to become cancerous[4]. There are several risk factors involved in CRC carcinogenesis including age, family history of colon cancer, smoking, obesity and diet[2,5]. Some epidemiological studies provide evidence that alterations in lipid and cholesterol metabolism occur during CRC progression[5-7]. Perturbation in lipid management is also associated with the development of CRC. A higher level of cholesterol and low-density lipoprotein (LDL) have been reported in most CRC patients, while high-density lipoprotein (HDL) has been reported as normal in 88.3% of CRC patients[8,9]. Thus, the genetic factors that affect lipid metabolism can be considered as predominant risk factors for CRC and therefore, can be targeted to improve cancer treatment response.

Apolipoprotein is a key gene in lipid metabolism. It controls cholesterol and triglyceride levels and facilitates the clearance of cholesterol and LDL from plasma[10]. Apolipoprotein E (apoE) is a complex glycoprotein synthesized mainly in the liver[11]. This protein is encoded by a polymorphic gene located on chromosome 19 with two common variants at two different codons, rs429358 at codon 112 and rs7412 at[12] codon 158. This variance results in three common alleles: allele ε2 (TT), which codes for the apoE2 isoform (has a cysteine residue at both positions), allele ε3 (TC), which codes for the most common isoform in humans (apoE3) (has a cysteine residue at codon 112 and an arginine at codon 158) and finally allele ε4 (CC), which codes for apoE4 (has an arginine residue at both positions)[13].

The substitution between cysteine and arginine in the gene encoding apoE has a pivotal role in the lipid binding activities of apoE. It has been found that apoE4 preferentially binds to very low-density lipoprotein (VLDL), whereas, both apoE2 and apoE3 prefer to bind to HDL[14]. Pathological processes of different diseases may be affected by the presence of a certain apoE isoform. For instance, apoE4 was examined as a risk factor for cardiovascular disease (CVD) and it is also reported as a strong genetic risk factor for Alzheimer disease (AD). On the contrary, ε2 allele is associated with lower risk of AD[15,16]. A growing body of evidence suggests a plausible role of *apoE* genotyping in cancer progression in general and in CRC specifically. However, in Saudi Arabia, less is known about the correlation between lipid metabolism and CRC. Therefore, the aim of this study was to analyze the genotype distribution and allele frequency among CRC patients in Jeddah, Saudi Arabia and to correlate these genetic variations with CRC prognosis.

MATERIALS AND METHODS

Subjects and samples: This case-control study was carried out during the period of September, 2018 to April, 2019. EDTA anticoagulated venous blood samples were collected from 131 Saudi participants aged from 30-70 years. The participants were divided into two groups: (1) CRC patient group (n = 66) who were recruited from the oncology center at King Abdullah Medical City (KAMC), Jeddah, Saudi Arabia and (2) healthy control group (n = 65) who were recruited from the blood bank unit at King Fahad General Hospital in Jeddah, Saudi Arabia. In this study, the diagnosis of CRC participants was performed by a consultant oncologist and confirmed by histopathological examination that divided the patients into low grades (n = 16) and high grades (n = 50), whereas, the diagnosis of healthy controls was based on physical examination and laboratory tests. All participants signed a consent form that follows the Declaration of Helsinki - Ethical Principles for Medical Research involving Human subjects and completed a questionnaire that contains physical and nutritional information. This research was approved by the permanent ethical research committee at King Abdulaziz University, Jeddah, Saudi Arabia (Reference number 191060335). The genomic deoxyribonucleic acid (gDNA) was extracted from peripheral blood leukocytes in whole blood samples using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The quality of each extracted DNA sample was assessed by measuring the absorbance at two wavelengths (260 and 280). All DNA samples were kept at temperature -20°C for future experiments.

Genotyping of *apoE* gene: The different *apoE* genotypes were determined by polymerase chain reaction (PCR) followed by restriction fragments length polymorphism (RFLP) assay. The experimental work of this study was conducted at the

Cancer and Mutagenesis Unit, King Fahd Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia. The PCR reaction was carried out in a 25 µL reaction that contains the following: 2 µL of (50 ng/µL) DNA, 12.5 µL PCR Master Mix (HotStart-IT® FideliTaq™ PCR Master Mix (2X), Affymetrix, USA), 8.5 µL RNase free water, 1 µL of (100 pmol/µL) of forward primer (5'ACAGAATTCGCCCCGGCCTGGTACAC-3'),1 µL of (100 pmol/µL) of the reverse primer (5'-TAAGCTTGGCA CGGCTGTCCAAGGA-3')[17]. The PCR thermocycler reaction condition involved an initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 65°C for 1 min and an extension at 72°C for 1 min. A final extension step was performed at 72°C for 5 min. To confirm successful amplification, PCR products were visualized by electrophoresis on 2% agarose gel containing ethidium bromide. Detection of different isoforms of the *apoE* gene was performed using FastDigest enzyme (*Hhal*, Thermo Scientific, USA). The digestion was performed according to the manufacturer's instructions without performing the inactivation step. The digested product was separated on 2% agarose gel at 85V for 30 min before being detected using a gel documentation system. To assess the qualification of RFLP, 10% of the PCR product samples from both patients and controls were selected randomly and sent to the Center of Excellence in Genomic Medicine Research (CEGMR) at King Fahd Medical Research Center, KAU, Jeddah, Saudi Arabia to confirm the results by DNA sequencing.

Statistical analysis: GraphPad Prism version 5.00 (San Diego California, USA) was used to perform the statistical analysis. Mann-Whitney test was used to compare physical characteristics regarding one parametric character. Contingency analysis by chi-square (χ^2) test was used to determine the different allele frequencies of the *apoE* gene by applying the Hardy-Weinberg equilibrium equation. The strength of association between genotyping of the *apoE* gene and risk of CRC was analyzed by Fisher's exact test by calculating the odds ratio, relative risk and 95% confidence interval. All values of $p<0.05$ were considered statistically significant. The data were expressed as mean±standard error of the mean (SEM).

RESULTS

Determination of the study participants' physical characteristics: The physical characteristics of patients and controls are shown in Table 1. Highly significant differences can be seen between controls and patients in weight and body mass index (BMI), with no obvious statistical difference in other characteristics. The comparison between males and females within the same group revealed that male controls showed highly significant difference in the height and waist to hip ratio (WHR) compared to female controls ($p<0.0001$) (Table 2), whereas the same comparison in patients revealed that male CRC patients had a significant difference in height only ($p<0.0001$) and weight ($p = 0.04$) (Table 3).

Genetic distribution of the *apoE* gene genotypes among control and patient groups: In the study population, for the *apoE* gene, three common alleles (ε2, ε3 and ε4) and four genotypes (E2/E3, E3/E3, E3/E4 and E4/E4) were identified. The distribution of *apoE* genotypes was calculated for controls and CRC patients using the Fisher's exact test. Of the 131 individuals comprising healthy controls and CRC patients combined, homozygous E4/E4 was the most common genotype, with 63.08% in controls and 51.51% in CRC patients.

Table 1: Physical characteristics comparison between controls and CRC patients

Physical characteristics	Groups	Numbers	Mean±SEM	p-value
Age (years)	Control	65	52.49±1.45	0.09
	Patients	66	55.73±1.51	
Height (cm)	Control	65	164.20±1.43	0.79
	Patients	66	165.10±1.15	
Weight (kg)	Control	65	82.29±1.87	0.001**
	Patients	66	73.17±1.94	
Body mass index (kg m^{-2})	Control	65	30.66±0.709	<0.0001***
	Patients	66	26.84±0.695	
Waist (cm)	Control	65	104.30±2.92	0.53
	Patients	66	100.30±2.43	
Hip (cm)	Control	65	109.60±2.10	0.93
	Patients	66	110.00±2.30	
Waist-to-hip ratio	Control	65	0.954±0.021	0.53
	Patients	66	0.917±0.017	

**p<0.01, ***p<0.001 were calculated by Mann-Whitney test and were considered significant, SEM: Standard error of mean

Table 2: Physical characteristics comparison between controls according to gender

Physical characteristics	Groups	Numbers	Mean±SEM	p-value
Age (years)	Male	43	51.51±1.84	0.219
	Female	22	54.41±2.32	
Height (cm)	Male	43	169.30±1.38	<0.0001***
	Female	22	154.30±1.94	
Weight (kg)	Male	43	85.09±2.12	0.056
	Female	22	76.82±3.46	
Body mass index (kg m^{-2})	Male	43	29.86±0.83	0.107
	Female	22	32.23±1.29	
Waist (cm)	Male	43	107.90±4.07	0.052
	Female	22	97.27±2.94	
Hip (cm)	Male	43	108.60±2.78	0.449
	Female	22	111.40±3.04	
Waist-to-hip ratio	Male	43	0.995±0.028	<0.0001***
	Female	22	0.875±0.017	

***p<0.001 was considered significant, SEM: Standard error of mean

Table 3: Physical characteristics comparison between patients according to gender

Physical characteristics	Groups	Numbers	Mean±SEM	p-value
Age (years)	Male	50	55.60±1.26	0.85
	Female	16	56.13±3.40	
Height (cm)	Male	50	167.70±1.26	<0.0001***
	Female	16	157.13±1.29	
Weight (kg)	Male	50	75.70±2.16	0.04*
	Female	16	65.25±3.69	
Body mass index (kg m^{-2})	Male	50	26.98±0.80	0.77
	Female	16	26.41±1.42	
Waist (cm)	Male	50	100.61±2.91	0.79
	Female	16	99.31±4.43	
Hip (cm)	Male	50	110.28±2.68	0.82
	Female	16	109.31±4.61	
Waist-to-hip ratio	Male	50	0.92±0.02	0.45
	Female	16	0.92±0.04	

*p<0.05,***p<0.001 were considered significant, SEM: Standard error of mean

Table 4: Comparison of *apoE* genotypes frequency between control and patients' groups using contingency analysis

		Frequencies		
Groups	*apoE* genotypes	Numbers	Percentage	Fisher's exact test p-value
Controls	E2/E3	24	36.92	0.286
	E4/E4	41	63.08	
	Total	65	100.00	
Patients	E2/E3	30	45.45	
	E3/E3	1	1.52	
	E3/E4	1	1.52	
	E4/E4	34	51.51	
	Total	66	100.00	
	apoE alleles			Chi-square (χ^2) p-value
Controls	ε2	24	18.46	0.20
	ε3	24	18.46	
	ε4	82	63.08	
	Total	130	100.00	
Patients	ε2	30	22.73	
	ε3	33	25.00	
	ε4	69	52.27	
	Total	132	100.00	

This was followed by the heterozygous E2/E3, which accounts for 36.92% in controls and 45.45% in CRC patients (Table 4).

Interestingly, in CRC patients only, two other genotypes for the *apoE* gene were observed alongside E2/E3 and E4/E4. Homozygous E3/E3 and heterozygous E3/E4 genotypes were

Table 5: Comparison of *apoE* genotypes distribution in controls and CRC patients regarding gender

apoE genotypes	Control males		Control females		Fisher's exact test p-value	Odds ratio (OR)	Relative risk (RR)
	Number	Percentage	Number	Percentage			
E2/E3	14	21.5	10	15.4	0.4162	0.5793 (0.2018-1.663)	0.7163 (0.3821-1.343)
E4/E4	29	44.6	12	18.5			
	CRC patient males		CRC patient females				
E2/E3	21	31.81	9	13.64	0.6988	Not applicable	Not applicable
E3/E3	1	1.52	0	0.00			
E3/E4	1	1.52	0	0.00			
E4/E4	27	40.90	7	10.61			

Table 6: Relationship between *apoE* genotypes and CRC prognosis

apoE genotypes	TNM stages	Frequencies		Chi-square (χ^2)	Chi-square (χ^2) p-value
		Number	Percentage		
E2/E3	I	4	13.33	4.236	0.895
	II	3	10.00		
	III	7	23.33		
	IV	16	53.33		
E4/E4	I	3	8.82		
	II	4	11.76		
	III	10	29.41		
	IV	17	50.00		

also found in CRC patients but at a lower percentage compared to E4/E4 and E2/E3, as illustrated by the incidence of 1.5% for each genotype (Table 4). On the other hand, the allele frequency for each allele (ε2, ε3 and ε4) was calculated by chi-square test. The allele frequency of both the controls and CRC patients was in agreement with the Hardy-Weinberg equilibrium (χ^2 = 3.192, degree of freedom (df) = 2 and p>0.05). The distribution of each allele was as follows: ε2 (18.46% in controls and 22.73% in CRC patients), ε3 (18.46% in controls and 25% in CRC patients) and ε4 (63.08% in controls and 52.27% in CRC patients). The frequencies revealed that allele's ε2 and ε3 are highly distributed in CRC patients, whereas allele ε4 is mostly distributed in healthy controls (Table 4). When a comparison was made between males and females in the control and CRC patient groups, the distribution of *apoE* genotypes was not statistically different between males and females in the control and patient group (p = 0.42 and p = 0.69), respectively (Table 5).

Relationship between *apoE* genotypes and CRC prognosis: The different genotypes of the *apoE* gene were distributed according to the clinical stages of the patients' CRC. Among the (n = 66) CRC patients, most of the patients that carry the E2/E3 genotype (n = 30) were clinically classified as stage I = 4, stage II = 3, stage III = 7 or stage IV = 16. On the other hand, patients with E4/E4 genotype (n = 34) were classified as follows: stage I = 3, stage II = 4, stage III = 10 or stage IV = 17. Although the results showed that most of the CRC patients carrying either E2/E3 or E4/E4 might have an increased risk of CRC progression compared with E3/E3 and E3/E4 carriers, the comparison made by chi-square test to reveal the risk association, showed a non-significant difference in risk between the two genotypes (E2/E3 and E4/E4) (χ^2 = 4.236, df = 9, p = 0.8952) (Table 6).

DISCUSSION

Worldwide, CRC is considered one of the deadliest cancers in both males and females. Several factors are associated with increased risk of CRC including metabolic syndromes. Many studies have indicated a direct effect of these syndromes on increasing risk of CRC[18]. The apoE is a multifunctional protein that plays a critical role in the regulation of lipoprotein metabolism[10]. Since lipid metabolism is closely related to CRC carcinogenesis, however, in Saudi Arabia, less is known about the correlation between lipid metabolism and CRC. Therefore, in the present study the genotypes of *apoE* gene were determined and their correlations with CRC risk were investigated. In this study, four genotypes (E2/E3, E3/E3, E3/E4 and E4/E4) were found to be distributed only in CRC patients and two genotypes (E2/E3 and E4/E4) in the healthy controls. The frequencies of the ε2 and ε3 alleles were found to be lower in CRC patients, whereas ε4 allele was more frequent in controls. However, none of the

genotypes or alleles were found to be correlated significantly with CRC risk in the studied population.

In agreement with the current findings, results from a meta-analysis study in 2014 that included eight prospective studies found no significant association between *apoE* polymorphism and CRC risk[19]. Moreover, another study by Kervinen *et al.*[20] found that patients with proximal colon cancer had a lower frequency of apolipoprotein E allele ε4 but higher protection against CRC formation compared to distal colon carcinoma. Furthermore, a study performed on male Japanese CRC patients suggested that altered lipid metabolism may be differentially associated with tumorigenesis in proximal and distal CRC, with a protective role of ε4 allele against distal and proximal CRC adenomas formation[21]. In other contexts, many studies have evaluated the correlation of the presence of specific *apoE* genotypes with carcinomas in different parts of the colon. A case-control study including 219 white Australian adults found a significant trend towards a lower risk for proximal (right-sided) cancers in patients with apolipoprotein ε4 (OR 0.64, 95% CI 0.31-1.33)[22]. Another study found that the presence of the E4/E4 genotype in healthy controls may have a protective effect against CRC progression as well as lowering lipid profile levels among patients, even those on lipid-rich diets[23]. It is noteworthy that the absence of the ε3 *apoE* allele was found to significantly increase the risk of colon cancer particularly in CRC patients older than 64 years (OR =1.88 95% CI 1.17-3.04, $p \leq 0.05$)[24]. In the present study, CRC patients showed a reduction in the frequency of ε3 but this was not significantly correlated with the risk of developing CRC. In contrast to the results in this the study, Watson *et al.*[25] found that *apoE* genotype can influence both CRC risk and prognosis of the existing disease in a gender-dependent manner. They found that patients with the E2/E3 genotype had an increased risk of colon cancer [odds ratio (OR) =1.91, 95% confidence interval 1.05-3.45]. When those patients were divided based on their gender, they found a highly significant association in men [odds ratio (OR) =2.71, 95% confidence interval 1.30-5.65] but no association in women [odds ratio (OR) =1.01, 95% confidence interval 0.37-2.77][25]. The data from the current study shed light on the relationship between *apoE* genotypes and CRC progression. The finding of this study could be useful in developing a laboratory panel for *apoE* mutations that might help in the diagnosis of CRC patients based on their lipid metabolism. The major limitation of this study is the small sample size and number of previous studies conducted on Saudi patients which might affect some of the statistical comparisons. Therefore, further studies are needed on larger number of populations to fully understand the pathways involved in lipid metabolism and lipid profile tests.

CONCLUSION

This current study is the first study that examines the role of *apoE* genotypes distribution in the risk of colorectal cancer in Saudi patients. The results of the study showed no differences in the frequency of *apoE* genotypes or alleles between the controls and CRC patients as well as no significant relation to CRC prognosis. Furthermore, the distribution of *apoE* genotypes between males and females CRC patients demonstrated a non-significant gender-based difference. The major limitation of this study is the small sample size and the small number of previous studies conducted on Saudi patients. Other future works are needed on larger number of samples and to investigate the different pathways involved in lipid metabolism and lipid profiling tests.

SIGNIFICANCE STATEMENT

Apolipoproteins particularly apoE are important in controlling lipid metabolism. Lipid metabolism is closely related to CRC carcinogenesis. Therefore, the determination of genetic variants of *apoE* gene might be important in revealing the response of CRC patients to drugs in the future based on their genotype's distribution. Hence, the current research identified the genotype distribution and allele frequency of *apoE* gene in CRC patients to draw a comprehensive picture about the genetic map of this gene in Saudi CRC patients and latterly, how this can affect their response to chemotherapy.

ACKNOWLEDGMENT

The study was self-funded by the postgraduate student, Manar F. Alharbi and Dr. Ayat B. Al-Ghafari.

REFERENCES

1. Bray, F., J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre and A. Jemal, 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: Cancer J. Clin., 68: 394-424.
2. Gandomani, H., S.M. Yousefi, M. Aghajani, A. Mohammadian-Hafshejani, A. Tarazoj, V. Pouyesh and H. Salehiniya, 2017. Colorectal cancer in the world: Incidence, mortality and risk factors. Biomed. Res. Ther., 4: 1656-1675.
3. Saudi Cancer Registry, 2018. Cancer incidence report Saudi Arabia 2015. Saudi Cancer Registry, National Health Information Center, Saudi Health Council, Kingdom of Saudi Arabia. https://nhic.gov.sa/eServices/Documents/E%20SCR%20final%206%20NOV.pdf

4. Van Lanschot, M.C.J., B. Carvalho, C. Rausch, P. Snaebjornsson and M. van Engeland *et al*., 2019. Molecular profiling of longitudinally observed small colorectal polyps: A cohort study. EBioMedicine, 39: 292-300.
5. Ganjali, S., B. Ricciuti, M. Pirro, A.E. Butler, S.L. Atkin, M. Banach and A. Sahebkar, 2018. High-density lipoprotein components and functionality in cancer: State-of-the-art. Trends Endocrinol. Metab., 30: 12-24.
6. Long, J., C.J. Zhang, N. Zhu, K. Du and Y.F. Yin *et al*., 2018. Lipid metabolism and carcinogenesis, cancer development. Am. J. Cancer Res., 8: 778-791.
7. Ding, X., W. Zhang, S. Li and H. Yang, 2019. The role of cholesterol metabolism in cancer. Am. J. Cancer Res., 9: 219-227.
8. Liao, F., W. He, C. Jiang, C. Yin and G. Guo *et al*., 2015. A high LDL-C to HDL-C ratio predicts poor prognosis for initially metastatic colorectal cancer patients with elevations in LDL-C. OncoTargets Ther., 8: 3135-3142.
9. Rodriguez-Broadbent, H., P.J. Law, A. Sud, K. Palin and S. Tuupanen *et al*., 2017. Mendelian randomisation implicates hyperlipidaemia as a risk factor for colorectal cancer. Int. J. Cancer, 140: 2701-2708.
10. Poirier, J., J. Miron, C. Picard, P. Gormley, L. Theroux, J. Breitner and D. Dea, 2014. Apolipoprotein E and lipid homeostasis in the etiology and treatment of sporadic Alzheimer's disease. Neurobiol. Aging, 35: S3-S10.
11. Karavia, E.A., D.J. Papachristou, I. Kotsikogianni, I. Giopanou and K.E. Kypreos, 2011. Deficiency in apolipoprotein E has a protective effect on diet-induced nonalcoholic fatty liver disease in mice. FEBS J., 278: 3119-3129.
12. Calero, O., R. Hortiguela, M.J. Bullido and M. Calero, 2009. Apolipoprotein E genotyping method by real time PCR, a fast and cost-effective alternative to the TaqMan® and FRET assays. J. Neurosci. Methods, 183: 238-240.
13. Nielsen, D.A., C.J. Spellicy, M.J. Harding and D.P. Graham, 2019. *Apolipoprotein* E DNA methylation and posttraumatic stress disorder are associated with plasma ApoE level: A preliminary study. Behav. Brain Res., 356: 415-422.
14. Saito, H., P. Dhanasekaran, F. Baldwin, K.H. Weisgraber, M.C. Phillips and S. Lund-Katz, 2003. Effects of polymorphism on the lipid interaction of human apolipoprotein E. J. Biol. Chem., 278: 40723-40729.
15. Eichner, J.E., S.T. Dunn, G. Perveen, D.M. Thompson, K.E. Stewart and B.C. Stroehla, 2002. Apolipoprotein E polymorphism and cardiovascular disease: A HuGE review. Am. J. Epidemiol., 155: 487-495.
16. Sorli, J.V., D. Corella, F. Frances, J.B. Ramirez, J.I. Gonzalez, M. Guillen and O. Portoles, 2006. The effect of the *APOE* polymorphism on HDL-C concentrations depends on the cholesterol ester transfer protein gene variation in a Southern European population. Clin. Chim. Acta, 366: 196-203.
17. Emi, M., L.L. Wu, M.A. Robertson, R.L. Myers and R.R. Hegele *et al*., 1988. Genotyping and sequence analysis of apolipoprotein E isoforms. Genomics, 3: 373-379.
18. Pirro, M., B. Ricciuti, D.J. Rader, A.L. Catapano, A. Sahebkar and M. Banach, 2018. High density lipoprotein cholesterol and cancer: Marker or causative? Progr. Lipid Res., 71: 54-69.
19. Tian, Y., J. Wang, Y. Ye, L. Sun and Y. Fan *et al*., 2014. Apolipoprotein E polymorphism and colorectal neoplasm: Results from a meta-analysis. PLoS ONE, Vol. 9. 10.1371/journal.pone.0102477.
20. Kervinen, K., H. Sodervik, J. Makela, J. Lehtola, M. Niemi, M.I. Kairaluoma and Y.A. Kesaniemi, 1996. Is the development of adenoma and carcinoma in proximal colon related to apolipoprotein E phenotype? Gastroenterology, 110: 1785-1790.
21. Shinomiya, S., J. Sasaki, C. Kiyohara, E. Tsuji and H. Inoue *et al*., 2001. *Apolipoprotein E* genotype, serum lipids and colorectal adenomas in Japanese men. Cancer Lett., 164: 33-40.
22. Butler, W.J., P. Ryan and I.C. Roberts-Thomson, 2001. Metabolic genotypes and risk for colorectal cancer. J. Gastroenterol. Hepatol., 16: 631-635.
23. Souza, D.R.S., M.A. Nakazone, M.A.S. Pinhel, R.M. Alvares and A.C. Monaco *et al*., 2009. Association between apolipoprotein E genotype, serum lipids and colorectal cancer in Brazilian individuals. Braz. J. Med. Biol. Res., 42: 397-403.
24. Slattery, M.L., C. Sweeney, M. Murtaugh, K.N. Ma and J.D. Potter *et al*., 2005. Associations between *apoE* genotype and colon and rectal cancer. Carcinogenesis, 26: 1422-1429.
25. Watson, M.A., L. Gay, W.S.L. Stebbings, C.T.M. Speakman, S.A. Bingham and A. Loktionov, 2003. Apolipoprotein E gene polymorphism and colorectal cancer: Gender-specific modulation of risk and prognosis. Clin. Sci., 104: 537-545.