

Research Article

Association of Apolipoprotein C3 -455T>C gene variant with Nonalcoholic Fatty Liver Disease in Obese Egyptians

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Citation: Elbaz R, Elshaer A, Sheriff M, Othma Y, Mahfouz M, et al. (2017) Association of Apolipoprotein C3 -455T>C gene variant with Nonalcoholic Fatty Liver Disease in Obese Egyptians. Int J Genom Data Min: IJGD-114. DOI: 10.29014/IJGD-114. 000014

Received Date: 02 November, 2017; **Accepted Date:** 22 November, 2017; **Published Date:** 29 November, 2017

Abstract

Background: Apolipoprotein C3 (APOC3) gene polymorphisms were reported to be associated with non-alcoholic fatty liver disease (NAFLD), hyper-triglyceridaemia, and insulin-resistance.

Objective: This study was undertaken to test the association of APOC3 gene variants with liver dysfunction, abnormal lipid profile or insulin resistance in obese Egyptian subjects.

Methods: The study was carried out on 100 unrelated obese Egyptians affected with NAFLD. These cases were compared to 83 normal weight healthy controls. All participants were subjected to an estimation of their body mass index (BMI) in addition to liver and renal functions and lipid profile. In addition, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed to detect APOC3 -455T>C (rs2854116) and APOC3 -482C>T (rs2854117) polymorphic genotypes.

Results: Cases showed a significantly higher frequency of the APOC3 -455 CC genotype than controls (p=0.0003, OR=5.33, 95% CI=2.2-12.7). Also, the allelic frequency of the rare APOC3 -455 C allele was significantly higher among cases than controls (p=0.0001, OR=2.35, 95% CI=1.5-3.6). On the other hand, cases showed a non-significant difference from controls regarding all APOC3 -482C>T genotypes and alleles. Although all obese cases were significantly showing affection of liver function, lipid profile and blood glucose levels compared to controls, they did not differ from each other in relation to their APOC3 genetic polymorphic types.

Conclusion: The polymorphism APOC3 -455T>C but not the APOC3 -482C>T in APOC3 gene was associated with NAFLD in Egyptian obese subjects. However, this polymorphism was not correlated to the degree of affection of liver function, lipid and glucose status.

Keywords: Apolipoprotein C-III; Genetic; Obesity; Polymorphism; Non-alcoholic Fatty Liver Disease

Introduction

One of the most common chronic liver disorders is the nonalcoholic fatty liver disease (NAFLD) [1] which characterized by hepatic steatosis in the absence of alcohol consumption or other liver disorders [2] Both environmental and genetic factors contribute to the process of steatosis, steatohepatitis (NASH) and fibrosis. [3] Risk factors include obesity and type 2 diabetes/insulin resistance. [4] Insulin resistance promotes peripheral adipose lipolysis, thereby increasing FFA flux to the liver, which drives hepatic triglyceride production [5]. Human studies have demonstrated peripheral adipose lipolysis, systemic free fatty acid levels, and de novo hepatic lipogenesis to be upregulated in subjects with NAFLD [6]. Fatty acid (palmitate) release from peripheral adipose deposits is increased approximately 35% in NAFLD patients compared to age, gender, and fat mass matched controls, and it accounts for approximately 60% of hepatic lipid in subjects with NAFLD. [6] De novo lipogenesis accounts for 25% of hepatic fat content in NAFLD subjects compared with 10% in obese hyper-insulinemic subjects and 5% in healthy individuals. [7]

In fact, a genetic factor underpinning NAFLD has been suggested by familial aggregation studies, [8] heritability studies, [9] candidate gene studies, [3] genome-wide scans [10] and expression studies. [11] The probing into the genetics of NAFLD will help in the identification of individuals at risk, understanding NAFLD pathogenesis and developing new therapies. APO lipoprotein (APO) C-III, a protein produced by the liver [12], is an essential constituent of VLDL and HDL [13]. Considering the inhibitory effect of APOC-III on lipoprotein lipase (LPL) activity and hepatic uptake of lipoproteins [14], reports of APOC-III gene variants have been proposed as being potentially responsible for the occurrence of lipoprotein lipid profile disturbances. Accordingly, numerous polymorphisms in the APOC-III gene have been identified. [15] The aim of this work is to investigate the association of APO lipoprotein C3 Gene variants with nonalcoholic fatty liver disease in Egyptian patients.

Subjects and Methods

This study has involved 100 subjects affected with NAFLD, recruited from the Department of Obesity and Diabetes; Internal Medicine Specialized Hospital, Mansoura University, Egypt. The patients were selected randomly of those being overweight or obese with a BMI at least > 27 and an ultrasound diagnosis of NAFLD after exclusion of other causes of steatohepatitis such as diabetes mellitus, hypothyroidism, nephrotic syndrome, pregnancy and alcohol intake. Their age mean±SD was 45.4±15.2 years. They were in the form of 40 (40%) males and 60 (60%) females. Cases were characterized according to the scores given by the World Health Organization (WHO), Adult treatment panels (ATP) and

International Diabetic Federation (IDF) [16].

For comparison, negative control group was selected including 83 healthy non-obese unrelated subjects. For all participants, the levels of total cholesterol, triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein (LDL-C), were determined by enzymatic methods. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), fasting and postprandial blood sugar, HbA1c and other parameters were carried out according to validated methods. DNA extraction was carried out for all participants using the generation DNA purification capture column kit (Gentra system, USA) which is based on a proprietary system that uses two reagents, a DNA purification solution and a DNA elution solution, along with a specially formulated purification matrix. SNPs in APOC3 gene, APOC3 -455T>C (rs2854116) and APOC3 -482C>T (rs2854117) were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR RFLP) method [17]. Primers used for DNA amplification were: forward: 5'-GGCTGTGAGAGCTCAGCCCT-3' and reverse: 5' TCACACTGGAATTCAGGCC-3'. The amplified 196 bp PCR product was digested with MspI enzyme to genotype polymorphism C-482T, and Fok I enzyme for SNP, APOC3 -455T>C (Fermentas, Fast Digest) by incubating at 37°C for 5 min followed by separation of fragments on 3% agarose gel. The digestion with MspI enzyme for -482C indicated by complete digestion into two bands of 143 and 53 bp whereas, "T" allele, shows two bands of 159 and 37 bp. Heterozygous -482C/T genotype was detected by four bands of 159, 143, 53 and 37 bp. Restriction digestion with FokI enzyme showed no digestion for -455 C allele and PCR amplicon (196 bp) was left undigested. PCR product was digested into two bands of 133 and 63 bp for -455T allele and for heterozygous and it results to three bands of 196, 133 and 63 bp.

Statistical analysis

Statistical analysis of data was done using the software statistical package SPSS program version 17. Student t-test was used to compare the numerical values related to lipid profile, other chemical parameters and body mass index whereas chi square, Fisher exact and odds ratio -with 95% confidence interval- were used to compare frequencies of different genotypes and alleles among cases and controls. Hardy-Weinberg equilibrium (HWE) law was used to test the concordance of expected genotype frequencies to the observed ones using the chi square test.

Results

Cases and controls showed a non-significant difference regarding their age and gender ratio. However, cases showed significant lower levels of hemoglobin, red cell count, platelet count, serum albumin, and HDL together with significant higher levels of white cell count, creatinine level, sGOT, serum bilirubin, fasting and postprandial blood sugar, HbA1C level, CPK, cholesterol, LDL and TG levels (Table 1).

	Cases	Controls	p
	100	83	
Male/Female	40/60	30/53	0.35
	Mean±SD	Mean±SD	
AGE (year)	45.45±15.29	45.55±15.53	0.964
HB (gram%)	11.31±1.74	13.37±1.93	.000**
RBCs (million/ml)	3.77±.61	4.77±.31	.000**
WBCs (thousand/ml)	6.28±2.31	5.46±1.34	.005*
Platelets (thousand/ml)	167.69±62.75	197.53±49.74	.001*
Creatinine (mg%)	.98±.25	.66±.26	.000**
sGPT (IU/L)	38.71±43.48	31.52±8.04	0.139
sGOT (IU/L)	43.07±38.97	29.98±6.71	.003*
Bilirubin (mg%)	1.29±1.29	.90±.14	.006*
Albumin (gram%)	3.81±.51	4.27±.49	.000**
FBS (mg%)	131.83±56.25	80.96±9.31	.000**
PBS (mg%)	223.23±114.65	127.81±23.39	.000**
HBA1c (gram%)	7.00±2.01	4.90±.38	.000**
CPK (IU/L)	156.75±53.03	75.94±13.41	.000**
Total Cholesterol (mg%)	230.37±48.58	82.89±16.23	.000**
LDL (mg%)	153.05±34.31	83.98±10.99	.000**
HDL (mg%)	54.89±11.44	60.96±6.99	.000**
TG (mg%)	131.43±72.92	78.49±14.87	.000**
sGPT: Serum glutamic pyruvic transaminase, sGOT: Serum glutamic oxaloacetic transaminase, FBS: fasting blood sugar, PBS: postprandial blood sugar, CPK: creatine phosphokinase, LDL= low-density lipoprotein cholesterol, HDL= high-density lipoprotein, TG= triglyceride, * p = <0.05 (significant), ** p= <0.001 (extremely significant)			

Table 1: Demographic, clinical and chemical data of cases of fatty liver compared to controls.

Regarding gene polymorphism, cases showed a significantly higher frequency of the APOC3 -455 CC genotype than controls (32% vs. 9.6%, p=0.0003, Odds ratio=5.33, 95% CI=2.2-12.7). Also, the frequency of the rare APOC3 -455 C allele was significantly higher among cases than controls (51% vs. 30.72%, p=0.0001, OR=2.35, 95% CI=1.5-3.6). Hardy Weinberg equilibrium testing showed a non-significant level among controls denoting that the observed genotype frequencies were conforming to the expected ones. On the contrary cases have shown a significant difference between the observed and expected frequencies due to an increase of the rare genotypes and alleles (Table 2). On the other hand, cases showed a non-significant difference from controls regarding all frequencies of APOC3 -482C>T genotypes (CT vs. CC, TT vs. CC and CT+TT vs. CC) as well as APOC3 -482 T vs. C allele frequency. Hardy Weinberg equilibrium showed a significant difference of the observed genotype frequencies from that the expected ones for both cases and controls probably due to increased rare allele homozygosity (TT) in both groups.

	Cases	Controls	p	Odds ratio	95% CI
	N (%)	N (%)			
C-482T					
CC	40 (40.0)	34 (41.0)	Reference		
CT	35 (35.0)	30 (36.1)	0.88	0.99	0.5-1.9
TT	25 (25.0)	19 (22.9)	0.92	1.12	0.5-2.4
CT+TT	60 (60.0)	49 (59.0)	0.98	1.04	0.6-1.9
C allele	115 (57.50)	98 (59.04)	Reference		
T allele	85 (42.50)	68 (40.96)	0.85	1.07	0.7-1.6
HWE	p<0.001**	p<0.001**			
T-455C					
TT	30 (30.0)	40 (48.2)	Reference		
TC	38 (38.0)	35 (42.2)	0.35	1.45	0.7-2.8
CC	32 (32.0)	8 (9.6)	0.0003**	5.33	2.2-12.7
TC+CC	70 (70.0)	43 (51.8)	0.018*	2.17	1.2-4.0
T allele	98 (49.0)	115 (69.28)	Reference		
C allele	102 (51.0)	51 (30.72)	0.0001**	2.35	1.5-3.6
HWE	p<0.05*	p>0.05			
HWE: Hardy Weinberg Equilibrium * p = <0.05 (significant), **p= <0.001 (extremely significant)					

Table 2: The distribution of genotype and allelic frequency of SNP, C-482T and APOC3 -455T>C of APOC3 gene between patients with nonalcoholic fatty liver disease and controls.

Comparing cases of fatty liver carrying the rare alleles of APOC3 gene (-482T and -455C) vs. others showed that the he distribution of BMI values, hematologic, liver function, renal function and lipid parameters were nearly similar denoting that

these polymorphisms might not be correlated to the degree of affection of liver function, lipid and other blood profiles (Table 3, 4).

BMI grade		APOC3 T-455C		APOC3 C-482T	
		TT	TC+CC	CC	CT+TT
		n (%)	n (%)	n (%)	n (%)
25.0–29.9	overweight	0(0)	1(1.4)	0 (0)	1 (1.7)
30.0–34.9	class I obesity	5(16.7)	19(27.1)	10 (25.0)	14 (23.3)
35.0–39.9	class II obesity	9(30.0)	20(28.6)	12 (30.0)	17 (28.3)
40.0–49.9	class III obesity	16(53.3)	22(31.4)	15 (37.5)	23 (38.3)
50.0–59.9	class IV obesity	0(0)	8(11.4)	3 (7.5)	5 (8.3)
Chi-Square (X ²)		p=0.112		p= 0.946	

Table 3: Distribution of BMI between cases carrying the rare allele of APOC3 -455T>C of APOC3 gene (TC+CC) vs. cases carrying the TT genotype.

	APOC3C-482T		P	APOC3 -455 T>C		p
	CC	CT+TT		TT	TC+CC	
	(n=40)	(n=60)		(n=30)	(n=70)	
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
HB (gram%)	11.17±1.86	11.39±1.67	0.54	11.25±1.85	11.33±1.70	0.84
RBCs (million/ml)	3.74±.58	3.80±.63	0.65	3.79±.57	3.77±.62	0.83
WBCs (thousand/ml)	5.84±1.79	6.57±2.58	0.13	6.28±3.05	6.27±1.94	0.98
Platelets (thousand/ml)	161.3±49.09	171.97±70.48	0.41	176.33±67.51	163.99±60.73	0.37
Creatinine (mg%)	.98±.19	.98±.29	0.96	.99±.30	.98±.23	0.69
sGPT (IU/L)	41.03±62.07	37.17±24.89	0.67	33.77±20.50	40.83±50.21	0.46
sGOT (IU/L)	42.30±51.17	43.58±28.57	0.87	44.37±31.81	42.51±41.86	0.83
Bilirubin (mg%)	1.32±1.58	1.28±1.07	0.87	1.05±.44	1.40±1.51	0.22
Albumin (gram%)	3.74±.52	3.86±.49	0.23	3.84±.55	3.80±.49	0.74
FBS (mg%)	131.1±54.17	132.30±58.04	0.92	130.97±39.78	132.20±62.23	0.92
PBS (mg%)	228.3±120.17	219.88±111.72	0.72	225.17±91.68	222.40±123.8	0.91
Total Cholesterol (mg%)	232.6±49.06	228.90±48.61	0.71	236.17±41.20	227.89±51.49	0.44
LDL (mg%)	156.3±33.66	150.92±34.85	0.45	156.20±30.78	151.70±35.84	0.55
HDL (mg%)	54.70±11.33	55.02±11.61	0.89	56.07±9.01	54.39±12.36	0.5

TG (mg%)	135.3±74.58	128.87±72.32	0.67	143.33±71.68	126.33±73.36	0.29
HBA1c (gram%)	7.08±1.91	6.95±2.08	0.75	6.96±1.48	7.02±2.20	0.9
CPK (IU/L)	152.6±47.28	159.55±56.75	0.52	166.97±56.80	152.37±51.13	0.21
BMI	40.30±6.76	40.03±6.41	0.84	39.68±5.31	40.34±7.00	0.64
sGPT: Serum glutamic pyruvic transaminase, sGOT: Serum glutamic oxaloacetic transaminase, FBS: fasting blood sugar, PBS: postprandial blood sugar, CPK: creatine phosphokinase, LDL= low-density lipoprotein cholesterol, HDL: high-density lipoprotein, TG: triglyceride.						

Table 4: Distribution of hematologic, chemical and lipid parameters between cases carrying the rare allele of C-482T of APOC3 gene vs. others.

Discussion

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in the world today. Hence, we were interested to study its underlying genetic basis -and to the first time- among Egyptian affected obese subjects. This study has shown that all patients were found to be also diabetic. This might confirm the hypothesis that insulin resistance might be a pathophysiological factor or “first hit” in the development of NAFLD [18]. These cases have shown a concurrent significant lower level of hemoglobin, red cell count, platelet count, serum albumin, and HDL together with a significant higher levels of white cell count, creatinine level, sGOT, serum bilirubin, fasting and post prandial blood sugar, HbA1C level, CPK, cholesterol, LDL and TG levels. Li MR et al. 2014 have reported that measurement values of BMI and lipid profile were significantly different between the control group and NAFLD group. They reported that obesity; elevated AST, low HDL, hypercholesterolemia, and hypertriglyceridemia were the most common characteristics in the NAFLD group [19]. The two SNPs in the promoter region of the APOC3 gene, rs2854117 and rs2854116 have been described to be associated with hypertriglyceridemia, metabolic syndrome and coronary artery disease [20]. More recently, these variants have been shown to be associated with the occurrence of NAFLD. Egyptian cases showed a significantly higher frequency of the APOC3 -455 CC genotype than controls. On the other hand, cases showed a non-significant difference regarding all APOC3 -482 C>T genotypes and alleles.

Interestingly, both polymorphisms showed no relation to patients’ levels of BMI, hematologic, liver function, renal function and lipid parameters. Similarly, Li et al study on Chinese patients showed that APOC3 -455 T>C genotypes were associated with NAFLD after adjusting for age, gender, and BMI [19]. On the other hand, the study of Yu et al. 2010 on Chinese Han Population showed the rare variant APOC3 haplotype was associated with the risk of hypertriglyceridemia in individuals without T2DM but not in those with it [21]. In agreement with our results, Puppala et al., 2014 have reported that among Southern Indian patients, APOC3 -455 T>C polymorphism was significantly associated with NAFLD with no significant association of the other -482C>T

polymorphism. Genotype APOC3-455 CC genotype was associated significantly with the elevated serum triglycerides in patients [22]. Also, Petersen et al. 2010 reported that NAFLD was found in 38 % of the Indian men in association with rare variant APOC3 alleles at one or both of these loci [23]. Researchers have proposed that the rare variant alleles led to increase in the amounts of APOC3 accompanied with inhibition of lipoprotein lipase activity and triglyceride clearance, resulting in hypertriglyceridemia due to increase in chylomicron remnants taken up by the liver resulting in NAFLD [24]. However, we might observe a different pattern related to ethnic origin particularly cases of European and American localities. So, to the contrary of our results, Richart and his colleagues 2010 found no relationship between APOC3 mRNA expression and triglycerides content in the livers of morbidly obese women of European descent. Also, they did not find any association between gene expression and plasma triglyceride concentrations or insulin-resistance index [25]. Also, Sentinelli et al., 2011 in their study among Southern European patients found no significant association between APOC3 polymorphisms and fatty liver disease, lipids, and insulin-resistance in obese subjects [26] Hyysalo et al., 2012 [27] reported a similar lack of association between the APOC3 gene polymorphisms and NAFLD on their investigation of Finnish population. In their study, individuals with and without the variant alleles (-455 C, - 482 T both) had similar amounts of liver fat, plasma APOC3 concentrations, serum triglycerides, HDL and levels of fasting plasma glucose, insulin and transaminases. Furthermore, other subsequent studies in Hispanic, European American, African American and European subjects have failed to confirm the association of APOC3 variants with NAFLD [28]. Similarly, the study reported by Niu et al., 2014 on Chinese Han patients did not find significant associations between these polymorphisms and the risk of NAFLD [29]. These variations might be due to studied population diversity, epigenetic and environmental impact on gene expression. Although our study might suffer some limitations related to its relatively small sample size, including a sample of patients all being obese and diabetic, it mostly discerns a conclusion of a positive association of NAFLD in Egyptian obese cases with the APO C3 -455T>C polymorphism and not with APOC3 -482C>T in APOC3 with no apparent impact on their levels of BMI, liver function and lipid profile.

Acknowledgement

We would like to thank the staff members of the Department of Endocrinology and Diabetes, Mansoura University Hospitals, Mansoura, Egypt for their sincere help and cooperation.

Conflict of Interest

This work is completely free from all issues related to conflict of interest.

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