LACK OF ASSOCIATION BETWEEN RS10755578 POLYMORPHISMS OF LIPOPROTEIN(A) GENE AND CORONARY ARTERY DISEASE IN IRANIAN POPULATION

MARYAM AHANI[1], SAYYED MOHAMMAD HOSSEIN GHADERIN[2], HOSEIN VAKILI[3], EZNOLLAH AZARGASHB[4], MANDANA HASANZAD[5]

[1]Department of Genetics, Tehran Medical Branch, Islamic Azad University, Tehran - [2]Medical Genetics Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran - [3]Department of Cardiology, Shahid Modarres Hospital, Tehran - [4]Assistant professor of School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran - [5]Medical Genomics Research Center, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran

ABSTRACT

Background*: Information confirming association or linkage between lipoprotein (a) gene polymorphisms and increased risk for coronary artery disease among different populations have remained rare. We investigated whether the polymorphism of rs10755578 of lipoprotein (a) gene was associated with elevated level of lipoprotein (a) and thus with cardiovascular risk among our population.*

Methods*: This case-control association study was conducted on 97 consecutive patients who were hospitalized at Shahid Modarres hospital in Tehran in 2014 with the diagnosis of coronary artery disease. Also, 94 healthy people have selected as the control. The gene polymorphism was examined by Taq Man Probe Real Time PCR technique.*

Results*: There was no significant difference in the frequency of rs10755578 of lipoprotein (a) gene between the case and healthy groups so that the frequency of wild genotype (CC) was 29.0% and 20.0% (as the reference, the frequency of homozygous genotype (CG) was 40.0% and 55.0% (p = 0.064), and the frequency of mutant genotype (GG) was 31.0% and 25.0% (p = 0.760), respectively. Comparing baseline data between the different genotypic patterns in case and control groups showed no differences that sex distribution, mean age, mean anthropometric indices, and mean serum biomarkers were not differ in different genotype subgroups between the cases and the healthy controls. In case group, the prevalence of cardiovascular risk factors was not significantly different between the genotype patterns.*

Conclusion*: Our study could not demonstrate the association between rs10755578 polymorphism of lipoprotein (a) and increased risk for coronary artery disease in Iranian population.*

Key words*: rs10755578, lipoprotein(a), coronary artery disease, Iranian population.*

Received *May 30, 2015;* ***Accepted*** *November 02, 2015*

Introduction

Lipoprotein (a) is an important subclasses of lipoproteins that has been recently suggested as a major risk factor for ischemic cardiovascular disorders[1,2,3]. Different physiological aspects of its function have been now revealed. The critical role of lipoprotein (a) in coagulation pathway seems plausible because of the high homology and similar structural condition between plasminogen and apolipoprotein (a) that its competing with plasminogen can lead to reduce fibrinolysis[4].

Also, lipoprotein (a) has been shown to be a trigger for secretion of Plasminogen activator inhibitor type 1 (PAI-1) antigen that cause thrombogenesis[5]. Furthermore, lipoprotein (a) can transports some atherogenic pro-inflammatory oxidized phospholipids to wall of the vessels leading vascular muscles proliferation[6]. In addition, this lipoprotein can also recruit inflammatory cells through interaction with angiogenesis[7]. Concluding the pointed mechanisms shows that increasing level of lipoprotein (a) can be a major risk factor for ischemic and inflammatory-based

cardiovascular and cerebrovascular events leading high mortality and morbidity. It should be noted that the level of lipoprotein (a) is mainly affected by different disease states and environmental factors. For instance, the level of this lipoprotein can be changed in the background of renal failure, prescribing lipid-reducing drugs, and slightly by environmental factors such as physical activity and dietary regimens[8,9,10]. Thus, lipoprotein (a) can be considered as a controllable cardiovascular risk factors.

Along with the impact of environmental factors affecting the level of lipoprotein (a), some gene polymorphisms have been recently focused involving the level and function of this particle. In fact, various types of gene polymorphisms and nucleotide sequence changes have been discovered both in the regulatory and coding sequences that affect lipoprotein (a) variability[11]. Some genetic studies could revealed association between the level of lipoprotein (a) variability and some gene polymorphisms on chromosome 6q27[12,13,14,15]. In overall, some minor and major allele variants have been recently proposed to be associated with elevated serum level of lipoprotein (a) as a major risk factor for coronary artery disease, however information confirming association or linkage between these gene polymorphisms and increased risk for coronary artery disease among different populations have remained rare. We investigated whether the polymorphism of rs10755578 of lipoprotein (a) gene was associated with elevated level of lipoprotein (a) and thus with cardiovascular risk among our population.

Materials and methods

Study population: This case-control association study was conducted on 97 consecutive patients (mean age 59.08 ± 8.75 years, 39% male) who were hospitalized at Shahid Modarres hospital in Tehran in 2014 with the diagnosis of coronary artery disease (according to WHO diagnostic criteria on coronary angiography and left ventriculography). In this study, angiographically identified stenoses >50% in one of the major coronary vessels at the time of the study were used to classify patients as having coronary involvement. Also, 94 healthy people (mean age 61.14 ± 11.07 years, 52% male) without history of ischemic heart diseases, evidences of coronary involvements or traditional cardiovascular risk profile including hypertension, hyperlipidemia, diabetes mellitus, smoking, opium use, family history of cardiovascular disorders or organic inflammatory disorders were randomly selected as the controls. Inclusion criteria for the study were age higher than 40 years and providing written informed consent. In addition, the exclusion criteria included history of pregnancy or breastfeeding, history of coronary angiography, heart failure, history of cardiovascular specific medications, or history of chronic systemic disorders such as liver or renal failure.

Study interventions: Before coronary angiography, 5cc of peripheral blood sample were collected in EDTA tubes from both cases and healthy controls. Baseline characteristics and clinical data of participants were collected by reviewing the recorded clinical charts or interviewing the patients if required. The cardiovascular risk factors have been also assessed including smoking history (patients smokes a tobacco product/products 6 times per week or totally 100 cigarettes), hypercholesterolemia (total cholesterol ≥200 mg/dl, HDL-cholesterol ≤40 mg/dl, triglycerides≥150 mg/dl), family history of CAD (first degree relatives before the age of 50 in men and 55 years in women), hypertension (systolic blood pressure≥140 mmHg and/or diastolic≥90 mmHg and/or on anti-hypertensive treatment), and diabetes mellitus (symptoms of diabetes plus plasma glucose concentration≥11.1 mmol/l or fasting plasma glucose≥110 mg/dl or 2-hp≥200 mg/dl). Genomic DNA was isolated from peripheral blood leukocytes according to a standard salting-out method using QIAamp DNA Micro kit (Qiagen Hilden Germany) consisted of the following steps: cell lysis by Sodium dodecyl sulfate (SDS) solution, denaturation of nucleoproteins and inactivation of cellular enzymes by Proteinase K, removal of contaminants with sodium chloride, and DNA precipitation by ethanol 70%. The purity of DNA samples was tested by NanoDrop Spectrophotometers (Thermo, 2000, USA) and gel electrophoresis. The gene polymorphism was examined by Taq Man Probe Real Time PCR technique using especial probes provided by Applied Biosystems Company. The distribution of genotypes was not significantly different from the Hardy-Weinberg equilibrium in both groups (p = 0.540).

Statistical analysis

Data were analyzed by the statistical software SPSS version 18 for windows (SPSS Inc., Chicago

IL). Quantitative variables were presented as mean ± standard deviation, and categorical variables were presented by absolute frequencies and percentages. Continuous variables were compared using t test. Whenever the data did not appear to have normal distribution or when the assumption of equal variances was violated across the group, Mann- Whitney U test was used. Categorical variables were compared using chi-square test. Fisher exact test was used when more than 20% of cells with expected count of less than 5 had been observed. P values of ≤ 0.05 were considered statistically significant.

Results

Comparing baseline variables between case group and control group (Table 1) showed significant differences in almost all baseline parameters including demographics, anthropometric indices, systolic and diastolic blood pressure and lipid profile. In this regard, the cases were older, had higher body mass index, had higher mean blood pressures, as well as had higher levels of fasting blood sugar, total cholesterol, and low density lipoprotein. There was no significant difference in the frequency of rs10755578 of lipoprotein (a) gene between the case and healthy groups so that the frequency of wild genotype (CC) was 29.0% and 20.0% (as the reference, the frequency of homozygous genotype (CG) was 40.0% and 55.0% (p = 0.064), and the frequency of mutant genotype (GG) was 31.0% and 25.0% (p = 0.760), respectively.

Factor	Case group (n = 97)	Control group (n = 94)	P-value
Age(years)	58.75±8.59	48.35±7.06	0.000
Male(%)	39%	52%	0.05
Height (cm)	160.0±11.46	161.48±9.27	0.000
Weight (kg)	69.40±14.22	65.71±11.17	0.048
BMI (kg/m)	27.28±6.76	25.13±3.13	0.005
SBP (mm Hg)	138.70±26.04	114.07±10.96	0.000
DBP (mm Hg)	84.57±12.64	75.29±6.97	0.000
TC(mg/dl)	173.46±32.55	172.04±19.26	0.710
TG(mg/dl)	153.21±68.67	112.21±58.40	0.000
FBS (mg/dl)	138.34±61.92	86.28±11.65	0.000

Table 1: Baseline data in case group and control group.

Also, comparing baseline data between the different genotypic patterns in case and control groups (Table 2) showed no differences that sex distribution, mean age, mean anthropometric indices, and mean serum biomarkers were not differ in different genotype subgroups between the cases and the healthy controls.

Factor	Case group (n = 97)				Control group (n = 94)			
	Wild	Heterozygous	mutant	p-value	Wild	Heterozygous	mutant	p-value
Age(years)	58.67± 8.90	59.20±8.74	58.23±0.49	0.89	49.78 ±7.06	48.51±7.56	46.78±5.72	0.38
Male(%)	17%	46%	35.8%	0.15	20%	57.1%	22.4%	0.89
Height (cm)	158.54±9.24	161.26±10.28	160.27±14.6	0.63	160.11±8.31	161.33±9.83	162.96±8.87	0.60
Weight (kg)	69.14±15.79	68.88±13.48	70.65±14.19	0.86	64.00±12.91	65.06±11.25	68.61±9.23	0.34
BMI (kg/m)	27.43±5.44	26.45±4.42	28.24±9.88	0.55	24.90±4.06	24.93±3.15	25.77±2.05	0.53
TC(mg/dl)	175.64±33.85	171.23±34.28	174.53±30.42	0.84	169.36±23.12	173.61±17.40	170.69±20.50	0.66
TG(mg/dl)	165.67±91.92	153.28±68.82	141.90±37.58	0.42	108.21±43.88	115.13±63.80	108.91±58.00	0.86
FBS (mg/dl)	157.46±85.5	129.56±48.34	131.80±49.45	0.15	87.10±14.60	85.30±10.94	87.82±10.78	0.18
HDL(mg/dl)	38.00±8.82	41.05±6.93	39.00±56.69	0.23	50.05±10.56	49.39±13.19	49.78±10.23	0.97
LDL(mg/dl)	102.82±17.88	99.00±24.03	107.13±29.48	0.39	86.85±35.29	90.53±23.05	88.02±26.86	0.85
SBP (mm Hg)	139.85±22.82	140.28±30.41	134.86±23.13	0.66	117.16±10.61	112.67±10.7	114.73±11.60	0.30

Table 2: Comparing baseline parameters between different genotypes of the polymorphism in case and control groups.

Moreover, in case group, the prevalence of cardiovascular risk factors was not significantly different between the genotype patterns. In this regard, in each wild, homozygous, and mutant genotype, high fasting blood sugar (> 110 mg/dl) was detected in 64%, 51%, and 43%, hypertriglyceridemia (> 200 mg/dl) in 17%, 12%, and 6%, hypercholesterolemia (> 200 mg/dl) in 14%, 15%, and 16%, high LDL level (> 130 mg/dl) in 10%, 15%, and 16%, low HDL level (< 35 mg/dl) in 32%, 10%, and 26%, systolic hypertension (> 140 mmHg) in 64%, 64%, and 40%, and diastolic hypertension (> 90 mmHg) in 32%, 36%, and 33%, respectively.

Discussion

As previously pointed, both genetic and environmental factors affect regulatory pathways of lipoprotein (a) and also process of atherosclerosis. Among recent gene polymorphisms that introduced

as inducer factors for elevating lipoprotein (a) level, the present study focused rs10755578 of lipoprotein (a) gene. Generally, the protein encoded by this gene is a serine inhibitory proteinase that limits activity of tissue-type plasminogen activator I. In fact, by attaching the wall of smooth muscles; the product of this gene can induce atherosclerotic lesions and thus promote thrombogenesis. The other hand, the elevation of this protein can linked to arterial atherosclerosis. In this regard, genome-wide association analyses revealed common DNA variants in lipoprotein (a) gene that contribute to plasma plasminogen level variation[16,17]. For instance, the presence of some gene polymorphisms such as rs9364559, rs10455872, and rs41272114 have shown to be associated with elevation or even reduction of lipoprotein (a) and thus can affect the risk for coronary artery disease[18,19,20].

However, a few studies have been published on the role of rs10755578 as an inducing polymorphic factor for coronary disease. We could not demonstrate the role of rs10755578 polymorphism in predicting increased risk for coronary artery disease. The previous studies resulted in contradictory results. In a study by Nicholls and colleagues[21] on North American population, the presence of this polymorphism was significantly associated with risk of coronary artery disease. In another study by Tregouet et al[22] in France population, it was reported to be associated with increased risk of coronary disease in the general population.

However, similar to our association study, Qi et al[23] could not show association between rs10755578 polymorphism and increased risk for coronary artery disease in American nation. Lv et al.[24] also indicated lack of association between this polymorphism and risk for coronary disease in Chinese population. Similarly, Zhi-Gen et al[25] could not show association between rs10755578 polymorphism and risk for coronary artery disease. Nicholls et al., determined that lipoprotein (a) polymorphism of rs10755578 as well as haplotypes correlated to plasma Lp(a) levels, however, this group did not observe any association of the LPASNPs/haplotypes with increased prospective MACE risk. Therefore, the existence of this polymorphism can be accompanied with triggering mechanisms of coronary atherosclerosis in some population, while may be neutral in some others. These paradoxical findings can be due to the difference in definitive inclusion and exclusion criteria of the studies, the difference if study planning, the difference in techniques used for genotyping and sequencing, as well as difference in specific genetic patterns in each population.

Despite lack of association between rs10755578 polymorphism and risk for cardiovascular ischemic events, the haplotypes this polymorphism with other polymorphisms in lipoprotein (a) were associated with the risk for coronary artery involvement. Tregouet et al.[22] identified the haplotypes CTTG and CCTC formed by rs2048327, rs3127599, rs7767084 and rs10755578 as risk haplotypes for coronary artery disease in six White populations. Sawabe et al[26] analyzed rs2048327 and rs10755578 in a large Japanese autopsy cases, and ascertained that haplotypes these two polymorphisms could work as risk factors for both coronary sclerosis and coronary artery disease. These result explained this fact that although the appearance of rs10755578 polymorphism alone may not be correlated to risk for coronary artery disease, but its haplotypes with other polymorphism can major role in this ischemic event.

In conclusion, our study could not demonstrate the association between rs10755578 polymorphism of lipoprotein (a) and increased risk for coronary artery disease in Iranian population. A few studies have been published on this role in various populations leading contradictory results that emphasizing further studies on the triggering role of this gene polymorphism and its haplotypes.

References

1) Nordestgaard BG, Chapman MJ, Ray K, Borén J, Andreotti F, Watts GF, Lipoprotein (a) as a cardiovascular risk factor: current status. Eur. Heart J., 2010, 23: 2844-2853.
2) Kamstrup PR, Tybjærg-Hansen A, Nordestgaard BG, Lipoprotein (a) and risk of myocardial infarction-genetic epidemiologic evidence of causality, Scand. J. Clin. Lab. Invest, 2011, 71(2): 87-93.
3) Danesh J, Collins R, Peto R, Lipoprotein (a) and coronary heart disease, Meta-analysis of prospective studies, Circulation, 2000, 102 (10): 1082-5.
4) McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, cDNA sequence of human apolipoprotein (a) is homologous to plasminogen, Nature, 1987, 330(6144): 132-7.
5) Sotiriou SN, Orlova VV, Al-Fakhri N, Ihanus E, Economopoulou M, Isermann B, Lipoprotein (a) in atherosclerotic plaques recruits inflammatory cells through interaction with Mac-1 integrin, FASEB J, 2006, 20 (3): 559-61.

6) Gouni-Berthold I, Berthold HK., Lipoprotein (a): current perspectives, Curr Vasc Pharmacol, 2011, 9(6): 682-92.
7) Tsimikas S, Witztum JL, The role of oxidized phospholipids in mediating lipoprotein (a) atherogenicity, Curr. Opin. Lipidol, 2008 19 (4): 369-77.
8) Christian Wilde, Hidden Causes of Heart Attack and Stroke: Inflammation, Cardiology's New Frontier. Abigon Press, 2003, pp. 182-183.
9) Takagi H, Umemoto T, Atorvastatin decreases lipoprotein (a): a meta-analysis of randomized trials, Int. J. Cardiol, 2012, 154 (2): 183-6.
10) Caplice NM, Panetta C, Peterson TE, Kleppe LS, Mueske CS, Kostner GM, Lipoprotein (a) binds and inactivates tissue factor pathway inhibitor: a novel link between lipoproteins and thrombosis. Blood 98, 2001, (10): 2980-7.
11) Pati U1, Pati N, Lipoprotein (a), atherosclerosis, and apolipoprotein (a) gene polymorphism. Mol Genet Metab. 2000, 71(1-2): 87-92.
12) Lackner C, Cohen JC, Hobbs HH, Molecular definition of the extreme size polymorphism in apolipoprotein (a), Hum Mol Genet. 1993; 2: 933-940.
13) Sandholzer C, Hallman DM, Saha N, et al. Effects of the apolipoprotein (a) size polymorphism on the lipoprotein (a) concentration in 7 ethnic groups. Hum Genet. 1991, 86: 607-614.
14) Utermann G. Genetic architecture and evolution of the lipoprotein (a) trait. Curr Opin Lipidol. 1999, 10: 133-141.
15) Broeckel U, Hengstenberg C, Mayer B, et al. A comprehensive linkage analysis for myocardial infarction and its related risk factors. Nat Genet. 2002, 30: 210-214.
16) DeMeester CA1, Bu X, Gray RJ, Lusis AJ, Rotter JI. Genetic variation in lipoprotein (a) levels in families enriched for coronary artery disease is determined almostentirely by the apolipoprotein (a) gene locus. Am J Hum Genet. 1995, Jan; 56(1): 287-93.
17) Ober C1, Nord AS, Thompson EE, Pan L, Tan Z, Cusanovich D, Sun Y, Nicolae R, Edelstein C, Schneider DH, Billstrand C, Pfaffinger D, Phillips N, Anderson RL, Philips B, Rajagopalan R, Hatsukami TS, Rieder MJ, Heagerty PJ, Nickerson DA, Abney M, Marcovina S, Jarvik GP, Scanu AM, Nicolae DL. Genome-wide association study of plasma lipoprotein (a) levels identifies multiple genes on chromosome 6q. J Lipid Res. 2009 May; 50(5):798-806. doi: 10.1194/jlr.M800515-JLR200. Epub 2009 Jan 5.
18) Song ZK1, Wu HD1, Cao HY1, Qin L1. The association between the LPA gene polymorphism and coronary artery disease in Chinese Han population. Biomed Res Int. 2014; 370670. doi: 10.1155/2014/370670. Epub 2014 Mar 25.
19) Santos PC, Bueno CT, Lemos PA, Krieger JE, Pereira AC. LPA rs10455872 polymorphism is associated with coronary lesions in Brazilian patients submitted to coronary angiography. Lipids Health Dis. 2014 Apr 29; 13: 74.
20) Kyriakou T1, Seedorf U1, Goel A1, Hopewell JC1, Clarke R1, Watkins H1, Farrall M2. PROCARDIS Consortium. A common LPA null allele associates with lower lipoprotein (a) levels and coronary artery disease risk. Arterioscler Thromb Vasc Biol. 2014 Sep; 34(9):2095-9. doi: 10.1161/ATVBAHA.114.303462. Epub 2014 Jun 12.
21) Nicholls SJ, Tang WH, Scoffone H, Brennan DM, Hartiala J, Allayee H, Hazen SL:Lipoprotein (a) levels and long-term cardiovascular risk in the contemporary era of statin therapy. J Lipid Res 2010; 51(10): 3055-3061.
22) Trégouët DA1, König IR, Erdmann J, Munteanu A, Braund PS, Hall AS, Grosshennig A, Linsel-Nitschke P, Perret C, DeSuremain M, Meitinger T, Wright BJ, Preuss M, Balmforth AJ, Ball SG, Meisinger C, Germain C, Evans A, Arveiler D, Luc G, Ruidavets JB, Morrison C, van der Harst P, Schreiber S, Neureuther K, Schäfer A, Bugert P, El Mokhtari NE, Schrezenmeir J, Stark K, Rubin D, Wichmann HE, Hengstenberg C, Ouwehand W; Wellcome Trust Case Control Consortium; Cardiogenics Consortium, Ziegler A, Tiret L, Thompson JR, Cambien F, Schunkert H, Samani NJ. Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery disease. Nat Genet. 2009 Mar; 41(3):283-5. doi: 10.1038/ng.314. Epub 2009 Feb 8.
23) Qi L, Ma J, Qi Q, Hartiala J, Allayee H, Campos H. Genetic risk score and risk of myocardial infarction in hispanics. Circulation 2011; 123: 374-380.
24) Lv X1, Zhang Y, Rao S, Liu F, Zuo X, Su D, Wang M, Xia M, Guo H, Feng D, Hong C, Li D, Ma W, Ouyang P, Li X, Feng X, Yang Y, Ling W, Qiu J. Lack of association between four SNPs in the SLC22A3-LPAL2-LPA gene cluster and coronary artery disease in a Chinese Han population: a case control study. Lipids Health Dis 2012 Oct 4; 11:128. doi: 10.1186/1476-511X-11-128.
25) Li ZG1, Li G, Zhou YL, Chen ZJ, Yang JQ, Zhang Y, Sun S, Zhong SL. Lack of association between lipoprotein (a) genetic variants and subsequent cardiovascular events in ChineseHan patients with coronary artery disease after percutaneous coronary intervention. Lipids Health Dis. 2013 Aug 27; 12:127. doi: 10.1186/1476-511X-12-127.
26) Sawabe M, Tanaka N, Arai T, Mieno MN, Ikeda S, Muramatsu M. Genetic polymorphisms of SLC22A3-LPL2-LPA gene cluster has direct and indirect effects on coronary atherosclerosis and coronary heart disease. HUGO J 2009; 4: 107

Corresponding author
SAYYED MOHAMMAD HOSSEIN GHADERIN
Medical Genetics Department School of Medicine, Shahid Beheshti University of Medical Sciences Tehran
(Iran)