Section: Biochemistry

Original Research Article

ANALYSIS OF LOW DENSITY LIPOPROTEIN RECEPTOR EXONS 12 AND 13 VARIATIONS IN RELATION TO LIPID PROFILE IN KERALA POPULATION

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ABSTRACT

Background: Introduction and aim: Coronary artery disease (CAD) is a multifactorial disorder resulting from the interaction of genetic and environmental influences. Thepresent study aimed to assess theassociation of Low-density of lipoprotein receptor (LDLR) exons 12 and 13variations in relation to lipid profile in coronary artery disease.
Materials and Methods: The study population consisted of 50 patients with coronary artery disease and 30 healthy control subjects. Fasting blood sample were collected for lipid profile analysis. Genomic DNA was extracted, amplified, and analyzed using an allele-specific polymerase chain reaction techniques to investigate LDLR gene polymrphisms.
Results: Lipid profile parameters showed a significant association ($p < 0.000$) with coronary artery disease. There was, nostatistically significant change in the allele and genotype frequencies of the LDLR SNPs rs688 and rs5925 between the case and control groups investigated.
Conclusion: The current investigation was unable to detect a significant connection between the LDLR gene SNPs rs688 and rs5925 with CAD.
Keywords: Coronary artery disease, LDLR gene, single nucleotide polymorphisms, allele-specific polymerized chain reaction, genotype frequencies, lipid profile.

INTRODUCTION

Coronary artery disease (CAD) is a multifactorial condition caused by the interplay of hereditary and environmental factors.[1] Common contributors to CAD are obesity, smoking, and, hypertension. The deposition of cholesterol in the blood vessel leads to loss of elasticity of blood vessel and hardening of the blood vessel. This leads to damage to the wall of the blood vessels and the development of atherosclerosis.[2] Further leads to inflammation. The low-density lipoprotein receptors (LDL-R) play a crucial role in eliminating the excess LDL-C.[3] This will help in regulating cholesterol metabolism. These LDL receptors were present in the liver where the cholesterol is stored after the metabolic processes. The absorption of LDL needs the presence of LDL-R receptors. LDL-R is basically a transmembrane glycol protein.[4]

Genetic studies reported numerous novel gene loci that are consistently linked with diseases such as coronary artery disease (CAD) and atherosclerosis. Mutations in the LDLR gene have been linked to familial hypercholesterolemia, according to the research.[5] As LDL-R has a greater ability in maintaining the cholesterol homeostasis, the patients with LDL-R gene mutation were at risk of developing atherosclerosis and subsequently cardiac diseases. The key portions of the LDL-R gene that will undergo mutation are exons, splicing sites, and promotor regions.[6] LDLRAP1 (LDLR receptor adapter protein), Apo B (LDL absorption and metabolism), and PCSK9 (proprotein convertase subtilisin/Kexin type 9 serine protease) have all been linked to familial hypercholesterolemia. LDL-C and coronary heart disease are linked to an LDLR exon 12 single nucleotide polymorphism (SNP) called rs688 that does not depend on gender (CAD).[7] However, the association between a genetic variation

in LDLR and CAD is not clear in the ethnic group of South Indian people. Thus, the present study was undertaken to investigate two LDLR gene polymorphisms, rs688 and rs5925, in CAD patients and normal controls from South India. Additionally, this research sought to determine the relationship between CAD and lipid profile parameters and the atherogenic index, as well as the gene polymorphisms rs688 and rs5925.

Aim and objectives: This research sought to determine the relationship between CAD and lipid profile parameters and the atherogenic index, as well as the gene polymorphisms rs688 and rs5925.

MATERIALS AND METHODS

The present study was a case-control study where 50 patients with coronary heart diseases and 30 healthy, age and gender-matched participants were included. The study protocol was approved by the institutional ethics committee of P K Das Institute of Medical Sciences. Written, voluntary informed consent was obtained from all the participants. Detailed clinical data were obtained by using the standard proforma of the hospital. Using an allele-specific PCR, a polymorphism in the LDLR-rs688[exon 12] and rs5925[exon 13] genes was found. Allele-specific PCR is a method that depends on the use of sequence-specific PCR primers to amplify test DNA only in the presence of the target allele in the sample. The primer3 software was used to construct primers for genotyping LDLR-rs688[exon 12] and rs5925[exon 13]. Following procedures were done for genetic analysis of SNPs of LDLR gene.:

1. Isolation of Genomic DNA- A total of 80 samples was used for the study (50 patients + 30 controls). DNA was isolated from 2ml of the peripheral blood samples in good quantity and quality from all the samples using QI Aamp DNA Blood Midi Kit (QIAGEN).
2. Quantification of the isolated DNA -All the isolated DNA samples was quantified in Flourometer. Concentration of the samples varied from 23ng/ul to 66ng/ ul.
3. Allele – Specific PCR – rs688[exon 12] -The region harboring the variant were successfully amplified through polymerase chain reaction in thermal cycler (Eppendorf) using the custom designed primers. For the SNP rs688, two sets of PCR were conducted with each control and patient samples. Primers F1 and R are used in the case of first set, while primers F2 and R used in the case of second set of PCR reactions. The PCR reactions could successfully amplify the 191 bp long PCR product.

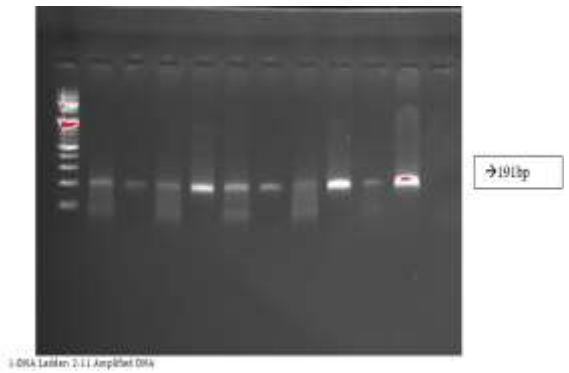

Figure 1: PCR amplification of the 191bp fragment harbouring the rs688 polymorphism

Figure 2: PCR amplification of the 176bp fragment harboring the rs5925[exon 13] polymorphism

In the representative gel image (Fig.2), the genotype of the first two samples (lanes 2 & 3 and lanes 4 & 5) should be CC, as the second primer pair (F & R2) amplified the 176bp product; not the first pair, in PCR. The genotype of the third sample (lanes 6 & 7), is TT, as the first primer pair (F & R1) produced the product. As both the primer pairs amplified the 176bp PCR product in the case of the fourth sample (lanes 8 & 9), both C and T alleles are present in the case of this sample; so, the genotype should be TC. The genotype of the last sample (Lanes 10 & 11) is again TT as the first primer set amplified the product. Likewise, genotypes of all the 80 samples were determined.

5ml of the fasting venous blood was collected following standard procedures from all the participants for biochemical analysis. In a fully automated chemistry analyzer, fasting plasma glucose, total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol (Direct) were determined. The atherogenic index was calculated by using the formula: log10 (TG/HDL-C).

Ethical considerations: The study protocol was approved by the institutional human ethics committee (IEC-NO/EC/AS/05/19).

RESULTS

Lipid profile of the study population

Total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol Totalwere estimated in the serum samples of case and control and is given in table 1. Significant difference (p=

0.00) was observed between cases and control groups studied.

Table 1: Levels of Lipid profile in case and control subjects

	Case	Control	p value
TC	231.06±28.0	178.7±15.6	0.000
HDL	36.4±2.9	46.1±4.7	0.000
LDL	157.5±26.8	113.4±11.13	0.000
TG	178.76±35.0	127.3±23.3	0.000

Association of TC, TG, HDL cholesterol and LDL cholesterol with CAD were determined by Chi square test and result are given in table 2. Significant association (p= 0.00) was observed between TC, TG, HDL cholesterol and LDL cholesterol and CAD.

Table 2: Association of TC, TG, HDL cholesterol and LDL cholesterol with CAD

Parameter		Control (N=30)	Test (N=30)	Chi Square	Df	P- Value
TOTAL CHOLESTEROL	<=200 mg/dl	30	14	39.273	1	0
	>200 mg/dl	0	36			
HDL CHOLESTEROL	<=40 mg/dl	6	46	42.725	1	0
	>40 mg/dl	24	4			
LDL CHOLESTEROL	<=100 mg/dl	5	1	5.814	1	0.016
	>100 mg/dl	52	49			
TG	<=150 mg/dl	29	1	71.694	1	0
	>150 mg/dl	1	49			

Genotype Study of rs688 polymorphism

Of the 50 patient samples tested for the rs688 polymorphism, 26 were heterozygous for the 'CT' genotype, 18 for the 'CC' genotype, and only six for the 'TT' genotype. Among the 30 control samples, 13 had the 'CT' genotype, 11 had the 'AA' genotype, and six had the 'TT' genotype. Table-3 summarizes the genotype and allele frequencies.

Table 3: Genotype and allele frequencies (in brackets) of rs688 SNP polymorphism in case and control groups

Rs688	N	Genotypes			Alleles	
		CC	CT	TT	C	T
Control	30	11 (0.37)	13 (0.43)	6 (0.2)	35 (0.58)	25 (0.42)
Cases	50	18 (0.36)	26 (0.52)	6 (0.12)	62 (0.62)	38 (0.38)
Total	80	29	39	12	97	63
		-0.36	-0.49	-0.15	-0.61	-0.39

However, no significant change in allele (p=0.64) or genotype (p= 0.57) frequencies was seen between the case and control groups investigated. The chi square test was used to determine the connection between the LDLR gene polymorphism rs688 and coronary artery disease (CAD) (Table 4). There was no significant connection between any of the three genotypes and CAD.

Table 4: Association of LDLR gene polymorphism rs688 with CAD

Genotype		CAD patients (N=50)	Controls (N=30)	Chi-Square	df	
C/C	N	31	20	0.004	1	0.952
	Y	17	12			
C/T	N	18	23	0.564	1	0.453
	Y	14	25			
T/T	N	25	43	0.941	1	0.332
	Y	6	6			

When we examine all the researched populations worldwide for the SNP polymorphism rs688 9exon 12), 'T' is the minor allele with a frequency of 0.28, according to the 1000 Genomes Project, a multinational research endeavor to create the most comprehensive database of human genetic diversity. This is mostly owing to the very high frequency of the 'C' gene, which is the ancestral allele, among African populations.

Present data of the South-Indian Kerala population is in agreement with the allele and genotype frequencies of the other South Asian populations. Among world populations, the closest one which showed a similar allele and genotype frequency as the Tamil

population is found to be the Indian Telugu population and is given in table 5.

Table 5: Allele and genotype frequencies of rs688 in Tamil population, in comparison with the other world populations. The closest population was found to be the Indian Telugu

Population	Allele Frequency		Genotype Frequency		
	C	T	CC	CT	TT
African	0.95	0.05	0.91	0.08	0.01
Latin American	0.54	0.46	0.27	0.54	0.19
East Asian	0.82	0.18	0.67	0.3	0.03
European	0.56	0.44	0.32	0.48	0.2
South Asian	0.62	0.38	0.39	0.45	0.16
Telugu	0.6	0.4	0.34	0.51	0.15
Kerala(Present study)	0.61	0.39	0.36	0.49	0.15

Genotype Study of rs5925[exon 13] polymorphism

Of the 50 patient samples checked for rs5925 polymorphism, 25 were found to be heterozygous with 'TC' genotype; 15 were 'TT' and 10 were 'CC'. Among the 30 control samples there were 14 'TC', 9'TT' and 7 'CC' genotypes. The genotype and allele frequencies are given in Table-6.

Table 6: Genotype and allele frequencies (in brackets) of rs5925 SNP polymorphism in case and control groups

Rs5925	N	Genotypes			Alleles	
		TT	TC	CC	T	C
Control	*30*	8 (0.30)	16 (0.47)	6 (0.23)	32 (0.53)	28 (0.47)
Cases	*50*	14 (0.30)	27 (0.50)	9 (0.20)	55 (0.55)	45 (0.45)
Total	*80*	22	43	15	87	73
		-0.3	-0.49	-0.21	-0.54	-0.46

However, there was no significant difference both in allele frequencies (p= 0.83) and genotype frequencies (p= 0.93) between cases and control groups studied. Chi square test was done to calculate the association of LDLR gene polymorphism rs5925 with CAD (Table 7). There was no significant difference association observed between the 3 genotypes with CAD.

Table 7: Association of LDLR gene polymorphism rs5925 with CAD

Allele/Genotype		CAD patients (N=50)	Controls (N=30)	Chi-Square	df	p Value
T/T	N	34	23	0.102	1	0.750
	Y	14	9			
T/ C	N	23	17	0.000	1	1.000
	Y	26	14			
C/C	N	24	39	0.124	1	0.724
	Y	9	8			

When we examine all the researched populations worldwide for the SNP polymorphism rs5925, 'C' is the minor allele with a frequency of 0.34, according to the 1000 Genomes Project, a multinational research endeavor to create the most comprehensive database of human genetic diversity. The South Indian Tamil population's current findings are consistent with the allele and genotype frequencies of other South Asian communities. Among international populations, the Sri Lankan Tamil population was determined to have the most comparable allele and genotype frequencies to the investigated Tamil population. [Table 8]

Table 8: Allele and genotype frequencies of rs5925 in the studied Tamil population, in comparison with the other world populations. The closest population was found to be the Sri Lankan Tamils

Population	Allele Frequency		Genotype Frequency		
	T	C	TT	TC	CC
African	0.85	0.15	0.72	0.26	0.02
Latin American	0.46	0.54	0.21	0.49	0.3
East Asian	0.78	0.22	0.62	0.32	0.06
European	0.55	0.45	0.31	0.48	0.21
South Asian	0.55	0.45	0.31	0.47	0.22
Sri Lankan Tamil	0.54	0.46	0.34	0.51	0.15
Kerala (Present study)	0.54	0.46	0.3	0.48	0.22

Association of TC, TG, HDL cholesterol and LDL cholesterol with 3 genotypes of LDLR gene polymorphism rs688 were determined by Chi square test and result are given in table 9. No significant association (p= 0.00) was observed between TC, TG, HDL cholesterol and LDL cholesterol with genotypes of rs688.

Table 9: Associations of TC, TG, HDL cholesterol and LDL cholesterol with LDLR gene polymorphism rs688

	CC			CT			TT		
	Chi-Square	df	p	Chi-Square	df	p Value	Chi-Square	df	p Value
TC	0.214	1	0.644	0.638	1	0.424	1.042	1	0.307
HDL	1.286	1	0.257	0.552	1	0.458	0.874	1	0.350
LDL	0.498	1	0.480	0.136	1	0.712	0.623	1	0.430
TG	0.765	1	0.382	0.294	1	0.588	1.118	1	0.290

NA- number insufficient

Association of TC, TG, HDL cholesterol and LDL cholesterol with 3 genotypes of LDLR gene polymorphism rs5925 were determined by Chi square test and result are given in table 10. No significant association (p= 0.00) was observed between TC, TG, HDL cholesterol and LDL cholesterol with genotypes of rs5925.

Table 10: Associations of TC, TG, HDL cholesterol and LDL cholesterol with LDLR gene polymorphism rs5925

	TT			TC			CC		
	Chi-Square	df	p Value	Chi-Square	df	p Value	Chi-Square	df	p Value
TC	0.215	1	0.643	1.024	1	0.312	0.487	1	0.485
HDL	1.752	1	0.186	0.664	1	0.415	0.298	1	0.585
LDL	0.934	1	0.334	0.119	1	0.730	0.556	1	0.456
TG	1.126	1	0.289	0.378	1	0.539	0.221	1	0.638

NA- number insufficient

DISCUSSION

Coronary artery disease (CAD) is a primary cause of morbidity and death globally and has developed into a significant public health burden in India.[6] Numerous studies have shown the importance of the lipid profile in the development of coronary artery disease. It was reported that increases in triglyceride (TG) and total cholesterol (TC) levels are significantly correlated with the risk of cardio vascular disease (CVD).[2] It was reported that increases in the low-density lipoprotein cholesterol (LDL-C) level could induce arteriosclerosis. However, the risk of cardiovascular disease (CVD) may be decreased in those with higher high-density lipoprotein cholesterol (HDL-C) levels.[4] In the present study we found that TC, TG and LDL cholesterol was significantly high whereas HDL cholesterol is significantly low in CAD patients compared to controls and we also found that high levels of TC, TG, LDL cholesterol and low levels of HDL cholesterol were significantly associated with CAD which is in agreement with the above-mentioned studies.

Recent genome-wide association studies have shown a significant link between common variants at the LDLR gene and a proatherogenic lipid profile and coronary artery disease.[7] Single nucleotide polymorphisms at the LDLR gene that contribute to inter-individual variance in blood lipid concentrations via genome-wide association analyses. Several SNPs within LDLR, including rs12983082, rs2738446, rs1799898, rs9789302, and rs5925, were shown to be in linkage disequilibrium with LDLR rs688C/T r2 > 0.8, however none were linked with plasma lipids, indicating that rs688 is the causal underlying polymorphism.[10]

In Indian patients, both the LDLR rs688 TT genotype and the LDLR T allele were linked with an increased vulnerability to CAD. It was reported that significant variation in genotype distribution between CAD patients and matched healthy controls.[8] But in our study no such significant difference in genotype and allele frequencies of rs688 SNP polymorphism among case and control groups were observed. In the current research, no such significant variation in genotype and allele frequencies of the rs5925 SNP polymorphism was found between case and control groups.

Earlier studies reported a minor variation in the LDLR rs688 (Asn591 ACC!ACT) gene has been linked to a 4–10% rise in plasma cholesterol levels in multiple different populations.[9] Mutations in the LDLR gene may result in an increase in plasma LDL levels, increasing one's risk of developing atherosclerosis and coronary heart disease. It has been reported that mutations in the LDLR gene cause familial hypercholesterolemia.[10]

The current investigation found no correlation between LDLR gene variations and lipid metrics. There might be many explanations for the absence of a connection between the LDLR gene SNP

polymorphism and coronary artery disease in this research. At first glance, the condition may seem to be complex, with separate causes. The LDLR-related pathways may account for just a portion of the illness. Numerous additional possible factors might exist in our samples. Second, genetic risk factors may be population-based. Positive results in one demographic may not be duplicated in a different one. Finally, the sample size we used (30 controls and 50 cases) was quite small. This may not be adequate statistical power. A more comprehensive investigation with a larger sample size is necessary to acquire more definitive conclusions.

CONCLUSION

Significant correlations were observed between coronary artery disease (CAD) and lipid profiles, specifically total cholesterol, triglyceride, LDL, and HDL levels. The analysis did not reveal a statistically significant link between LDLR gene polymorphisms and CAD or lipid measures.

Regarding genetic diversity, the rs688 genotype and allele frequencies in the studied Kerala population closely matched the Indian Telugu group, whereas the rs5925 frequencies in the Kerala population were most comparable to those of Sri Lankan Tamils.[1,2]

REFERENCES

1. Esmaeili, N. A., & Ahmadi, K. J. Lipid abnormalities in urban population of Rafsanjan (Rafsanjan coronary risk factors study phase 1).2004.
2. Fu, Y., Katsuya, T., Higaki, J., Asai, T., Fukuda, M., Takiuchi, S., ... & Ogihara, T. A common mutation of low-density lipoprotein receptor gene is associated with essential hypertension among Japanese. Journal of human hypertension. 2001; 15(2), 125-130.
3. Jha, C. K., Mir, R., Khullar, N., Banu, S., & Chahal, S. M. S. LDLR rs688 TT genotype and T allele are associated with increased susceptibility to coronary artery disease—A case-control study. Journal of cardiovascular development and disease. 2018; 5(2), 31.
4. K Jha, C., Mir, R., Elfaki, I., Banu, S., & Chahal, S. M. S. Ldlr Gene Polymorphisms (Rs5925 and Rs1529729) Are Associated with Susceptibility to Coronary Artery Disease in a South Indian Population. Medical Sciences. 2019;7(7), 80.
5.
6. Karimi, F., Rayani, M., Akbarzade, S., Tahmasebi, R., Khakzade, M., & Arab,
7. J. The prevalence of hyperlipidemia in persons over 19 years of Bushehr in 1378. Iran South Med J. 2000; 3(2), 98-106.
8. Strøm, T. B., Tveten, K., Laerdahl, J. K., & Leren, T. P. Mutation G805R in the transmembrane domain of the LDL receptor gene causes familial hypercholesterolemia by inducing ectodomain cleavage of the LDL receptor in the endoplasmic reticulum. FEBS open bio. 2014 4, 321-327.
9. Teslovich, T. M., Musunuru, K., Smith, A. V., Edmondson, A. C., Stylianou, I. M., Koseki, M., ... & Dominiczak, A. F. Biological, clinical and population relevance of 95 loci for blood lipids. Nature. 2010; 466(7307), 707-713.
10. Topol, E. J., Smith, J., Plow, E. F., & Wang, Q. K. Genetic susceptibility to myocardial infarction and coronary artery disease. Human molecular genetics.2006; 15(suppl_2), R117-R123.
11. Zhao, X., Wang, D., & Qin, L. Lipid profile and prognosis in patients with coronary heart disease: a meta-analysis of prospective cohort studies. BMC cardiovascular disorders.2021; 21(1), 1-15.
12. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2014;63(25 Pt B):2889–2934.
13. Kruth HS. Lipoprotein cholesterol and atherosclerosis. Curr Mol Med. 2001 Dec;1(6):633-53.
14. Llorente V, Badimon L. Bases celulares y moleculares de la acumulación de colesterol en la pared vascular y su contribución a la progresión de la lesión aterosclerótica [Cellular and molecular bases of cholesterol accumulation in the vascular wall and its contribution to the progression of atherosclerotic lesion]. Rev Esp Cardiol. 1998 Aug;51(8):633-41.
15. Franceschini, N., Muallem, H., Rose, K. M., Boerwinkle, E., & Maeda, N. (2009). Low density lipoprotein receptor polymorphisms and the risk of coronary heart disease: the Atherosclerosis Risk in Communities Study. Journal of thrombosis and haemostasis : JTH, 7(3), 496–498.
16. Rader DJ, Cohen J, Hobbs HH. Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. J Clin Invest. 2003;111:1795–1803.
17. Soutar AK, Naoumova RP. Mechanisms of disease: genetic causes of familial hypercholesterolemia. Nat Clin Pract Cardiovasc Med. 2007;4:214–225.
18. Cohen JC, Boerwinkle E, Mosley TH, Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med. 2006;354:1264–1272.
19. Kathiresan S, Melander O, Anevski D, Guiducci C, Burtt NP, Roos C, Hirschhorn JN, Berglund G, Hedblad B, Groop L, Altshuler DM, Newton-Cheh C, Orho-Melander M. Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med. 2008;358:1240–1249.