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Original Article

Genetics Section

Assessment of Coronary Artery Disease and Lipoprotein Lipase Gene Polymorphism in Bengali Population by PCR based RFLP Analysis- A Retrospective Study

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ABSTRACT

Introduction: Coronary Artery Disease (CAD) is a leading cause of death in most countries as well as developing countries like India and incidence of susceptibility to CAD is associated with increased frequencies of particular Single Nucleotide Polymorphisms (SNPs) located in Lipoprotein Lipase (LPL) gene.

Aim: To evaluate the association of LPL gene polymorphisms variation namely LPL-*HindIII*, LPL-*PvuII* and LPL-*Ser447Ter* with CAD in Bengali population by Polymerase Chain Reaction (PCR) based Restriction Fragment Length Polymorphism (RFLP).

Materials and Methods: A retrospective case-control study was conducted at RG Kar Medical College and Hospital, Kolkata, West Bengal, India from April 2016 to October 2016. The study included 100 patients suffering from CAD and 100 controls from healthy populations were taken and Deoxyribonucleic Acid (DNA) extraction followed by genotyping was done by PCR based RFLP study. Hardy-Weinberg equilibrium for genotypic frequencies were analysed followed by goodness-of-fit Chi-square (χ^2) test to check the equilibrium of the SNP alleles.

Results: Total 100 patients, 57 males and 43 females with mean age 61.42±9.34 years suffering from CAD were cases and 100 patients were controls, 61 males and 39 females with mean age 49.37±10.21 years. It was found that genotypic frequencies for *HindIII*, *PvuII* and *MnlI* polymorphism of LPL gene were not deviated from the Hardy-Weinberg expectations in both control and cases groups. We could not find any significant association of the *HindIII*, *PvuII* and *Ser447Ter* polymorphisms of the LPL gene with occurrence of CAD in target population after appropriate data analysis using SPSS and MedCalc software.

Conclusion: Association and prediction of susceptibility patterns in ethnic population may require a prospective study involving higher number of cases which subsequently leads to possible pharmacogenomic utility on a broader perspective. Although authors did not get any statistically significant association between CAD and the genes of interest but these findings would lead to a better understanding of the condition and open up more avenues for study, treatment and prevention of CAD.

Keywords: Polymerase chain reaction, Restriction endonuclease, Restriction fragment length polymorphism, Single nucleotide polymorphisms

INTRODUCTION

The Coronary Artery Disease (CAD) is a major health problem and a major cause of death in most countries [1,2]. This disorder happens to be a prominent health problem in India and its incidence is growing in developing countries as well [3]. Although controversies are there, numbers of studies have established that there are increased frequencies of particular Single Nucleotide Polymorphisms (SNPs) which increase the susceptibility of CAD in the general population [4-15].

Lipoprotein Lipase (LPL) gene, chromosomally located at 8p22, spans over 30kb and is divided into 10 exons showing *HindIII* polymorphism of intron 8 and *PvuII* polymorphism of intron 6 has an association with elevated triglyceride level [16,17]. The *Ser447* Stop mutation has been identified at 635 base pair downstream from the *HindIII* polymorphism. This mutation is a consequence of a C to G transversion at nucleotide 1595 in exon 9, which converts the serine 447 codon (TCA) to a premature termination codon (TGA). Recent studies have suggested that risk of CAD is decreased by *Ser447* Stop polymorphism, which underlies a metabolic state of increased HDL cholesterol levels and decreased triglyceride levels. Thus, development of atherosclerosis and subsequently CAD should have protected by *Ser447* Stop polymorphism [18,19]. A study on an Indian population carried out by Ashok Kumar M, et al., did not find any significant association of LPL *HindIII* polymorphism with CAD [20], this lack of correlation has also been observed in Tunisian and Saudi population [1,21]. Therefore, we were intended to execute a study focusing the Bengali population in a tertiary care hospital to evaluate if LPL gene polymorphisms correlate with CAD.

Authors had evaluated a group of independent and well defined subjects in the present study giving importance to the LPL genes, LPL-*HindIII*, LPL-*PvuII* and LPL-*Ser447*Ter polymorphisms in CAD subjects.

MATERIALS AND METHODS

A retrospective case-control study was conducted in a tertiary care hospital (RG Kar Medical College and Hospital, Kolkata, West Bengal, India) over a period of six months, from April 2016 to October 2016, for its performance and final report submission. Patients with present/past history of CAD who attended the Department of Cardiology formed the study population. The study followed the guidelines of Helsinki declaration 2004 [22] and prior approval from the ICMR-STS project and the Institutional Ethics committee (Indian Council of Medical Research- Short Term Studentship (ICMR-STS) Id: 2016-0173, IEC: 21.04.15) were taken before commencing the present study.

Inclusion criteria: Patient who has attained 18 years and can give a valid written consent with confirmed diagnosis of CAD by angiography were included in the present study as cases. Controls were selected from a healthy population. Lipid profile of these subjects was performed to screen for any deviation from reference range.

Exclusion criteria: Subjects with negative angiographic findings, type-A personality, suffering from any metabolic disorder like Type 2 Diabetes Mellitus (T2DM), dyslipidemia, addiction of alcohol or smoking are established as risk factors of CAD were strictly excluded from the study. First degree relatives of the cases were excluded from the control population.

Study Procedure

Hundred patients suffering from CAD and 100 control populations from the same geographical area of more or less similar socio-economic status irrespective of their age and sex were taken after strictly following the inclusion and exclusion criteria. About 2 mL of blood using Ethylene Diamine Tetraacetic Acid (EDTA) as an anticoagulant was collected from all the subjects and were stored at -20°C till further procedure of genetic study. Lipid profile was performed from serum samples collected at least after 12 hours of fasting.

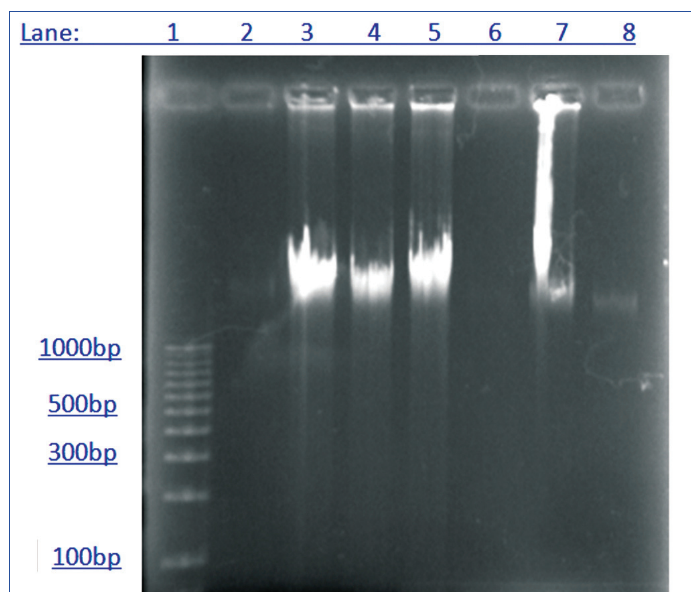
Polymerase Chain Reaction

DNA extraction: The genomic DNA was extracted from the whole blood using EDTA as anticoagulant according to the method of Blin and Stafford (1976) with few modifications [23].

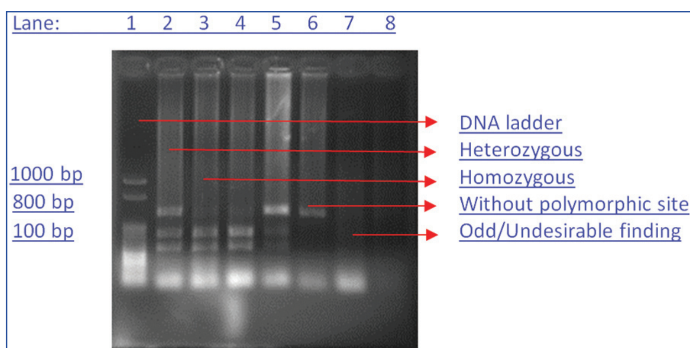
Buffers used: Lysis buffer (pH 8.0), TE buffer (pH 7.4), 50X TAE, 6X gel loading buffer. The quality of DNA was verified by ratio of the absorbance at 260 nm and 280 nm. Integrity of genomic DNA was assayed by electrophoresing the extracted DNA in 1% agarose gel.

PCR reaction: The PCR reaction was carried out using the reagents of HiMedia DNA amplification kit. The primers were purchased from Integrated DNA Technology. The master mix was prepared by mixing the 10X DNA amplification buffer (500 mM KCl, 100 mM Tris HCl, 15 mM $MgCl_2$),) 2.5 µM dNTP mix, 20 µM forward primer, 20 µM reverse primer, Molecular grade water, Taq DNA polymerase as follows: Total reaction volume of 25 µL.

PCR-RFLP: Genotyping was performed via amplification of the polymorphic regions by polymerase chain reaction found in introns 8, 6 and 9 followed by digestion of these amplified fragments with *HindIII* (Cleavage of the sequence between 5'-A|A G C T T-3'), *PvuII* (Cleavage of the sequence between 5'-CAG|CTG-3') and *MnlI* (Cleavage of the sequence between 5'-CCTCN7|-3') Restriction Endonucleases (RE) respectively. Image after restriction enzyme digestion [Table/Fig-1,2].



[Table/Fig-1]: Genomic DNA (Lane: 3, 4 and 5) Lane:1-DNA ladder, Lane: 2, 6 and 8- No findings, Lane: 7 Odd/Undesirable finding.



[Table/Fig-2]: After Restriction Endonucleases (RE) digestion.

Amplification protocol

1. *HindIII*

The region in intron 8 containing the *HindIII* polymorphism was amplified using the following primer pair [24]: LPL-H1 5'-TGA AGC TCA AAT GGA AGA GT-3'; LPL-H2 5'-TCA AAG CAA ATG ACT AAA-3'. Amplification protocol included 35 cycles, the amplified product was 770 bp in size (Denaturation- 94°C, 1 min; Annealing-53°C, 45 sec; Extension- 72°C, 1 min; Final extension- 7 min).

2. *PvuII*

The amplification of the *PvuII*-containing site (intron 6) will use the following primer pair [24]: LPL-P1 5'-ATG GCA CCC ATG TGT AAG GTG-3'; LPL-P2 5'-GTG AAC TTC TGA TAA CAA TCT C-3'. Amplification protocol included 30 cycles, the amplified product was 440 bp in size (Denaturation-94°C, 20 sec; Annealing-50°C, 30 sec; Extension-72°C, 20 sec; Final extension-7 min).

3. *Ser447*

The amplification of the *Ser447*Stop mutation containing site was amplified by using the following primer pair [19]: M1:5'- CAT CCA TTT TCT TCC ACA GGG -39; M2: 59-AGT CTG GTG AGC ATT CTG GGC TA -3'. The amplified product was 488 bp in size. Amplification protocol included Initial denaturation- 94°C, 5 minutes; Followed by- 30 cycles of: Denaturation- 94°C, 1 minute; Annealing- 57°C, 60 seconds; Extension- 72°C, 60 seconds; Final extension- 72°C, 10 minutes.

RFLP Analysis

Restriction digestion of PCR product for RFLP analysis:

Restriction reaction was done with the help of *HindIII*, *PvuII* and *MnlI* restriction enzymes (RE) purchased from FERMENTA GROUP SRL Company. These enzymes recognise G/T, C/T and C/G respectively. The reaction condition was as follows:

Total Reaction volume- 10 µL: Molecular grade water-1.5 µL, Assay buffer- 1 µL, RE Enzyme- 0.5 µL, PCR DNA- 7 µL were mixed and incubated for 2 hours 30 minutes at 37°C.

Agarose gel electrophoresis of the restriction fragments: The presence of the restriction site produces fragments of 600 and 170 bp in case of *HindIII* digestion, while the presence of the sites for *PvuII* yields fragments of 330 and 110 bp. A 488 bp PCR product of LPL gene contains two restriction sites for *MnlI*, of which one is attributable to *Ser447* stop mutation due to polymorphism. Digestion of the PCR product results in 3 identifiable fragments of 285, 246, and 203 bp. In all the cases digested products were captured on 2% agarose gel for improved resolution.

STATISTICAL ANALYSIS

Data analysis was done after completion of data collection for all patients using windows based Statistical Package for the Social Sciences (SPSS) version 17.0 and MedCalc version 11.3 software. The Hardy-Weinberg equilibrium for genotypic frequencies was analysed followed by goodness-of-fit Chi-square (χ^2) test to check the equilibrium of the SNP alleles [25-27].

RESULTS

Hundred patients, 57 males and 43 females with mean age 61.42±9.34 years suffering from CAD as cases and 100 controls, 61 males and 39 females with mean age 49.37±10.21 years populations from the same geographical area of more or less similar socio-economic status irrespective of their age and sex were recruited for this study. Finally, 87 cases and 82 controls gave expected electrophoretic patterns for the three restriction enzymes unanimously.

The RFLP profile of the study population: Restriction enzyme is capable to distinguish two individual alleles. These allelic forms with their zygosity and different genotypic distribution among the cases and controls after digestion with respective enzymes are described in [Table/Fig-3].

Group	Restriction enzyme	Homozygous (+/+)	Heterozygous (+/-)	Homozygous (-/-)
Case (N=87)	*HindIII* (T/G)	46	37	04
	PvuII (T/C)	28	41	18
	MnlI (G/C)	04	27	56
Control (N=82)	*HindIII* (T/G)	49	31	02
	PvuII (T/C)	26	38	18
	MnlI (G/C)	03	24	55

[Table/Fig-3]: Different genotype distribution among study population after digestion with respective enzymes.
+: polymorphic site, -: without polymorphic site

The allelic frequencies (T=0.741 and G=0.259) and genotypic frequencies (T/T=0.549, T/G=0.384 and G/G=0.067) of cases in comparison to the allelic frequencies (T=0.7866 and G=0.2134) and genotypic frequencies (T/T=0.6187, T/G=0.3357 and G/G=0.0456) of controls as shown in [Table/Fig-4,5] by *HindIII* digestion of LPL gene shows no statistical significance owing to much more p-value than 0.05 between each frequencies.

Gene	Genotype distribution			Allele frequency	
	T/T	T/G	G/G	T allele frequency (p*)	G allele frequency (q*)
HindIII					
Cases (87)	46 (52.87%)	37 (42.53%)	04 (4.6%)	0.741	0.259
Controls (82)	49 (59.76%)	31 (37.8%)	02 (2.44%)	0.7866	0.2134
PvuII					
Cases (87)	28 (32.18%)	41 (47.13%)	18 (20.69%)	0. 557	0.443
Controls (82)	26 (31.71%)	38 (46.34%)	18 (24.39%)	0.549	0.451
MnlI					
Cases (87)	04 (4.6%)	27 (31.03%)	56 (64.37%)	0.201	0.799
Controls (82)	03 (3.66%)	24 (29.27%)	55 (67.07%)	0.183	0.817

[Table/Fig-4]: Genotype distribution and allele frequency of the *HindIII, PvuII* and *Ser447*Ter in cases and controls.
*p and q are frequencies of the two alleles in a single locus

Variables	Allelic frequency		Genotypic frequency		
	T	G	T/T	T/G	G/G
Case (87)	0.741	0.259	0.549	0.384	0.067
Control (82)	0.7866	0.2134	0.6187	0.3357	0.0456
Chi-square test	χ^2=0.266 df=1		χ^2=0.581 df=1	χ^2=0.243 df=1	χ^2=0.0722 df=1
p-value	0.6060		0.4459	0.6218	0.7882

[Table/Fig-5]: Showing *HindIII* digestion-difference of allelic and genotypic frequency between cases and control with goodness-of-fit Chi-square (χ^2) test.

Hence, it was concluded that there was no significant association of *HindIII* LPL gene polymorphism with CAD in this study group. Authors also found that the allelic frequencies (T=0.557 and C=0.443) and genotypic frequencies (T/T=0.310, T/C=0.494 and C/C=0.196) of cases in comparison to the allelic frequencies (T=0.549 and C=0.451) and genotypic frequencies (T/T=0.301, T/C=0.495 and C/C=0.204) of controls as shown in [Table/Fig-4,6] by *PvuII* digestion of LPL gene showed no statistical significance owing to much more p-value than 0.05 between each frequencies. Hence, it was concluded that there was no significant association of *PvuII* LPL gene polymorphism with CAD in the present study group.

Variables	Allelic frequency		Genotypic frequency		
	T	C	T/T	T/C	C/C
Case (87)	0.557	0.443	0.310	0.494	0.196
Control (82)	0.549	0.451	0.301	0.495	0.204
Chi-square test	χ^2=0.00252 df=1		χ^2=0.00161 df=1	χ^2=0.198 df=1	χ^2=0.00390 df=1
p-value	0.9599		0.9680	0.6566	0.9502

[Table/Fig-6]: Showing *PvuII* digestion-difference of allelic and genotypic frequency between cases and control with goodness-of-fit Chi-square (χ^2) test.

Once again allelic frequencies (G=0.201 and C=0.799) and genotypic frequencies (G/G=0.04, G/C=0.321 and C/C=0.639) of cases in comparison to the allelic frequencies (G=0.183 and C=0.817) and genotypic frequencies (G/G=0.034, G/C=0.299 and C/C=0.667) of controls as shown in [Table/Fig-4,7] by *MnlI* digestion of LPL gene shows no statistical significance owing to much more p-value than 0.05 between each frequencies. Hence, it was concluded that there was no significant association of *MnlI* LPL gene polymorphism with CAD in this study group.

Variables	Allelic frequency		Genotypic frequency		
	G	C	G/G	G/C	C/C
Case (87)	0.201	0.799	0.04	0.321	0.639
Control (82)	0.183	0.817	0.034	0.299	0.667
Chi-square test	χ^2=0.0103 df=1		χ^2=0.0404 df=1	χ^2=0.0203 df=1	χ^2=0.0486 df=1
p-value	0.9192		0.8407	0.8866	0.8255

[Table/Fig-7]: *MnlI* digestion-difference of allelic and genotypic frequency between cases and control with goodness-of-fit Chi-square (χ^2) test.

DISCUSSION

Variation is the key factor in the evolution of population. Charles Darwin's (1859) "The Origin of Species by Means of Natural Selection", scientifically documented this variation in population. Variation is generally reflected in phenotypic polymorphism contributed by genetic differences. However, every genetic variation may not be reflected in phenotypic forms [28].

The Human Genome Project (HGP) has revealed that huge differences of nucleotide sequence exist in the different individuals and population in the same chromosomal location. If a difference is common and significant (>1%) in a population, it is defined as polymorphism [25]. Thus during the sequencing of human genome several polymorphisms in the level of single nucleotide, were indentified and designated as SNP. The inherited difference in DNA sequences, may contribute to phenotypic variation, anthropometric variation, and risk of diseases. Genome-wide linkage analysis and positional cloning have identified hundreds of genes for human diseases (Online Mendelian Inheritance in Man database- OMIM database) [29], but nearly all are rare conditions in which mutation of a single gene is necessary and sufficient to cause disease. For common diseases genome-wide linkage studies have had limited success. One promising approach is to systematically explore the limited set of common gene variants for association with disease [30,31]. In the human population most variant sites are rare, but the small number of common polymorphisms explains the bulk of heterozygosity [32]. As these common variants are responsible for most heterozygosity in a population, it will be important to assess their potential impact on variation of phenotypic trait.

Both Celera and International Human Genome Sequence Consortium (IHGSC) estimated that the frequency of SNPs in each individual is 8×10^{-4} per nucleotide or about one SNP every 1.25 kb [33]. There are 3.1 million SNPs recognised in the human genome [34]. As tools for understanding human variation, SNP can be used for gene mapping, definition of population, structure and performance of functional studies as well as disease association. SNPs are the most common (every 100-300 bases, 97% in non coding and 3% in coding region) genetic difference among people [35,36]. A SNP occurs when a point mutation happens in the genome, converting one nucleotide into another. If the mutation is in the reproductive cells of an individual then one or more of the offsprings might inherit the mutation, the SNP may eventually become established in the population after many generations [37].

The CAD is a major health problem and a major cause of death in most countries [1,2]. This disorder happens to be a prominent health problem in India, and its incidence is growing in developing countries as well [3]. There are several methods available for study of SNP and DNA sequence polymorphism like Denaturing High Pressure Liquid Chromatography (DHPLC), DNA hybridisation, Single-Stranded Conformational Polymorphism (SSCP), Restriction Fragment Length Polymorphism (RFLP) and direct DNA sequencing [38]. Restriction fragment length polymorphism is the one of the most popular methods for the study of the SNPs. Donnis-Keller H et al., first described the RFLP map of the entire human genome [39]. There are vast numbers of SNPs giving rise to RFLP in human genome. According to SNP Group (2001), there is 1,00,000 or more RFLP site available in the human genome [40].

Association of allelic and genotypic frequencies of LPL gene with CAD is not a unanimous finding by all the researchers throughout the globe as it depends on different populations. Peacock RE et al., performed a study involving 87 CAD patients and 93 healthy controls of Sweden and they did not find any impact of *HindIII*, *PvuII* and *Serine447*-Stop polymorphisms of the LPL gene locus with the progression of atherosclerosis [24]. In accordance with these authors, Al-Jafari AA et al., also came to a conclusion that there are no significant associations of the said three polymorphism of LPL gene with CAD recruiting 120 CAD subjects and 65 controls from Saudi populations [1]. Involving 475 CAD subjects among fair skinned population Wang XL et al., did not find any association of *HindIII* polymorphism with the severity of CAD but got a significant association of *PvuII* polymorphism [41].

In the present study, the genotypic frequencies for *HindIII*, *PvuII* and *MnlI* polymorphism of LPL gene are not deviated from the Hardy-Weinberg expectations in both control and CAD groups.

In case of *HindII* LPL gene polymorphism authors got allelic frequencies (T=0.741 and G=0.259) and genotypic frequencies (T/T=0.549, T/G=0.384 and G/G=0.067) of cases along with allelic frequencies (T=0.7866 and G=0.2134) and genotypic frequencies (T/T=0.6187, T/G=0.3357 and G/G=0.0456) of control group. Through performing Chi-square test authors did not get any positive statistical significance (p-value >0.05) involving case and control group owing to χ^2=0.266, p-value=0.6060 between allelic and χ^2=0.581, p-value=0.4459, χ^2=0.243, p-value=0.6218 and χ^2=0.0722, p-value=0.7882, respectively between genotypic frequencies of T/T, T/G and G/G. This type of negative association of *HindIII* LPL gene polymorphism with CAD as we found in Bengali population is in accordance with the findings in Swedish and Saudi population respectively as described by Al-Jafari AA et al., and Peacock RE et al., [1,24]. The finding of Wang XL et al., regarding the *HindIII* polymorphism of LPL gene in case of CAD also supports these type of findings [41].

Authors obtained allelic frequencies (T=0.557 and C=0.443) and genotypic frequencies (T/T=0.310, T/C=0.494 and C/C=0.196) of cases in comparison to the allelic frequencies (T=0.549 and C=0.451) and genotypic frequencies (T/T=0.301, T/C=0.495 and C/C=0.204) of controls by digesting PCR product of LPL gene with *PvuII*. Authors did not receive any statistical significance even in this polymorphism by doing Chi-square test and getting the results as follows χ^2=0.00252, p-value=0.9599 between allelic frequencies (T/C) and χ^2=0.00161, p-value=0.9680, χ^2=0.198, p-value=0.6566 and χ^2=0.00390, p-value=0.9502 respectively for T/T, T/C and C/C genotypic frequencies. This finding ropes the result of Al-Jafari AA et al., Peacock RE et al., but not in favour of the results specified by Wang XL et al., [1,24,41].

Mattu RK et al., described these three polymorphisms previously and he found that *HindIII* polymorphism is most significantly associated with CAD than *PvuII* polymorphism but polymorphism of *Ser447*Ter is not associated involving a population of 235 CAD, 124 minimal CAD and 111 severe CAD subjects [42]. Jemaa R et al., in an Etude Cas Temoinsurl' Infarctus du Myocarde (ECTIM) study linking 614 patients and 733 controls stated that the Odds of *HindIII* and *PvuII* positive polymorphic sites are significantly associated with CAD or its severity but the no relation of *Ser447*Ter [43].

For *Ser447*Ter polymorphism (G/C) involving 87 cases of CAD and 82 controls authors got the allelic frequencies (G=0.201 and C=0.799) and genotypic frequencies (G/G=0.04, G/C=0.321 and C/C=0.639) of cases in comparison to the allelic frequencies (G=0.183 and C=0.817) and genotypic frequencies (G/G=0.034, G/C=0.299 and C/C=0.667) of controls by *MnlI* digestion of LPL gene. Statistically authors found χ^2=0.0103, p-value=0.9192 between allelic frequencies as well as χ^2=0.0404, p-value=0.8407, χ^2=0.0203, p-value=0.8866 and χ^2=0.0486, p-value=0.8255 by doing Chi-square test. All the frequencies in every case shows no statistical significance as they all have higher p-values (p-value >0.05). This finding is in accordance to results obtained by Jemaa R et al., and Mattu RK et al., and also by a meta-analysis done by Xie L and Li YM involving 14 case control studies [42-44].

Limitation(s)

The major limitation of this study was the sample size. Due to the time constraint by ICMR-STS project , the sample size was not calculated by performing the formal statistical tests. Authors depended upon different studies by researcher at that time and accordingly included 100 subjects for each cases and controls. At this time authors are unable to confirm the findings by doing Sanger's sequencing.

CONCLUSION(S)

There was no association of the *HindIII*, *PvuII* and *Ser447*Ter polymorphisms of the LPL gene with occurrence of CAD in the study population. Higher numbers of samples may be required to establish an association in a prospective study. However, this study has a huge scope in not only formulating the aetiopathogenesis of disease but also in prediction of susceptibility patterns in ethnic population and subsequently a possible pharmacogenomic utility on a broader perspective.

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REFERENCES

[1] Al-Jafari AA, Daoud MS, Mobeirek AF, Al Anazi MS. DNA polymorphisms of the lipoprotein lipase gene and their association with coronary artery disease in the saudi population. Int J Mol Sci. 2012;13(6):7559-74.
[2] Manochehri M, Moghadam AJ. Studying the relation of postprandial triglyceride with Coronary Artery Disease (CAD). Med Arch. 2016;70(4):261-64.

[3] Spurthi KM, Kumar GR, Kumar GK, Chiranjeevi P, Cingeetham A, Srilatha G, et al. MMP 1 circulating levels and promoter polymorphism in risk prediction of coronary artery disease in asymptomatic first degree relatives. Gene. 2016 Sep 28.
[4] Wang Y, Lu Z, Zhang J, Yang Y, Shen J, Zhang X, et al. The APOA5 rs662799 polymorphism is associated with dyslipidemia and the severity of coronary heart disease in Chinese women. Lipids Health Dis. 2016;15(1):170.
[5] Khan S, Phulukdaree A, Ramkaran P, Moodley D, Chuturgoon AA. The Arg72 variant of the p53 functional polymorphism (rs1042522) is associated with coronary artery disease in young South Africans of Indian ancestry. Gene. 2016;593(2):261-64.
[6] Kastelein JJ, Groenemeyer BE, Hallman DM, Henderson H, Reymer PW, Gagne SE, et al. The Asn9 variant of lipoprotein lipase is associated with the -93G promoter mutation and an increased risk of coronary artery disease. The Regress Study Group. Clin Genet. 1998;53(1):27-33.
[7] Chen ML, Zhao H, Liao N, Xie ZF. Association between paraoxonase 2 Ser311Cys polymorphism and coronary heart disease risk: A meta-analysis. Med Sci Monit. 2016;22:3196-201.
[8] Chen QJ, Lai HM, Zhao L, Ma YT, Li XM, Zhai H, et al. Association between the NFKB1-94ins/del ATTG Polymorphism (rs28362491) and coronary artery disease: A systematic review and meta-analysis. Genet Test Mol Biomarkers. 2016;20(3):105-11.
[9] Chen Y, Lin M, Liang Y, Zhang N, Rao S. Association between Apolipoprotein B Xbal polymorphism and coronary heart disease in han Chinese Population: A meta-analysis. Genet Test Mol Biomarkers. 2016;20(6):304-11.
[10] Chen YY, Wang BN, Yu XP. Correlation between the 677C>T polymorphism in the methylene tetrahydrofolate reductase gene and serum homocysteine levels in coronary heart disease. Genet Mol Res. 2016;15(1).
[11] Su GB, Guo XL, Liu XC, Cui QT, Zhou CY. Association between interleukin-17A polymorphism and coronary artery disease susceptibility in the Chinese Han population. Genet Mol Res. 2016;15(3).
[12] Qin LM, Qin GM, Shi XH, Wang AL, Zuo H. Association between matrix metalloproteinase-9 rs3918242 polymorphism and development of coronary artery disease in a Chinese population. Genet Mol Res. 2016;15(2).
[13] Boghdady A, Arafa UA, Sabet EA, Salama E, El Sharawy A, Elbadry MI. Association between rs1761667 polymorphism of CD36 gene and risk of coronary atherosclerosis in Egyptian population. Cardiovasc Diagn Ther. 2016;6(2):120-30.
[14] Pereira A, Palma Dos Reis R, Rodrigues R, Sousa AC, Gomes S, Borges S, et al. Association of ADAMTS7 gene polymorphism with cardiovascular survival in coronary artery disease. Physiol Genomics. 2016 Sep 9:physiolgenomics 00059 2016.
[15] Jung JH, Song GG, Kim JH, Seo YH, Choi SJ. Association of factor XIII Val34Leu polymorphism and coronary artery disease: A meta-analysis. Cardiol J. 2016;24(1):74-84.
[16] Chamberlain JC, Thorn JA, Oka K, Galton DJ, Stocks J. DNA polymorphisms at the lipoprotein lipase gene: Associations in normal and hypertriglyceridaemic subjects. Atherosclerosis. 1989;79(1):85-91.
[17] Daoud MS, Ataya FS, Fouad D, Alhazzani A, Shehata AI, Al-Jafari AA. Associations of three lipoprotein lipase gene polymorphisms, lipid profiles and coronary artery disease. Biomed Rep. 2013;1(4):573-82.
[18] Stocks J, Thorn JA, Galton DJ. Lipoprotein lipase genotypes for a common premature termination codon mutation detected by PCR-mediated site-directed mutagenesis and restriction digestion. J Lipid Res. 1992;33(6):853-57.
[19] Groenemeijer BE, Hallman MD, Reymer PW, Gagne E, Kuivenhoven JA, Bruin T, et al. Genetic variant showing a positive interaction with beta-blocking agents with a beneficial influence on lipoprotein lipase activity, HDL cholesterol, and triglyceride levels in coronary artery disease patients. The Ser447-stop substitution in the lipoprotein lipase gene. REGRESS Study Group. Circulation. 1997;95(12):2628-35.
[20] AshokKumar M, Veera Subhashini NG, Kanthimathi S, SaiBabu R, Ramesh A, Cherian KM, et al. Associations for lipoprotein lipase and peroxisome proliferator-activated receptor-gamma gene and coronary artery disease in an Indian population. Arch Med Res. 2010;41(1):19-25 e1.
[21] Jelassi A, Jguirim I, Slimani A, Najah M, Hamda KB, Addad F, et al. Association between variants of lipoprotein lipase and coronary heart disease in a Tunisian population. Pathol Biol (Paris). 2012;60(3):180-84.
[22] World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. J Int Bioethique. 2004;15(1):124-29.
[23] Blin N, Stafford DW. A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res. 1976;3(9):2303-08.
[24] Peacock RE, Hamsten A, Nilsson-Ehle P, Humphries SE. Associations between lipoprotein lipase gene polymorphisms and plasma correlations of lipids, lipoproteins and lipase activities in young myocardial infarction survivors and age-matched healthy individuals from Sweden. Atherosclerosis. 1992;97(2-3):171-85.
[25] Snustad DP, Simmons MJ. Population Genetics. Principle of Genetics. 5th ed. New York: Wiley; 2010. Pp. 709-29.
[26] Snustad DP, Simmons Mj. Population Genetics. Principles of Genetics. 5th ed. New York: John Wiley & Sons, Inc.; 2010. Pp. 710-14.
[27] Snustad DP, Simmons MJ. Testing Genetic Hypothesis. Principles of Genetics. 5th ed. New York: John Wiley & Sons, Inc; 2010. Pp. 52-55.
[28] Snustad DP, Simmons MJ. Evolutionary Genetics. Principles of Genetics. 5th ed. New York: Wiley; 2010. Pp. 730-56.
[29] Online Mendelian Inheritance in Man [database on the Internet]. Johns Hopkins University. 1995. Available from: http://www.ncbi.nlm.nih.gov/omim.
[30] Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science. 1996;273(5281):1516-17.
[31] Collins FS, Guyer MS, Charkravarti A. Variations on a theme: Cataloging human DNA sequence variation. Science. 1997;278(5343):1580-81.
[32] Lander ES. The new genomics: global views of biology. Science. 1996;274(5287):536-33.
[33] Sudbery P, Sudbery I. The sequence of human genome. Human Molecular Genetics. 2nd ed2002. Pp. 87-136.
[34] Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature. 2007;449(7164):851-61.
[35] Rifai N, Warnick GR. Nucleic Acid Techniques. In: Burtis CA AE, Bruns DE, editor. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th edition ed. New Delhi: Saunders, Elsevier; 2006. Pp. 1407-09.
[36] Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, et al. Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat Genet. 1999;22(3):231-38.
[37] Brown TA. Genomes. 2nd ed. UK: John Wiley and Sons; 2002.
[38] A K, S S. The Single Nucleotide Polymorphism Database (dbSNP) of Nucleotide Sequence Variation. In: Book NH, editor: NCBI; 2007. Pp. 5.1-5.29.
[39] Donis-Keller H, Green P, Helms C, Cartinhour S, Weiffenbach B, Stephens K, et al. A genetic linkage map of the human genome. Cell. 1987;51(2):319-37.
[40] Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature. 2001;409(6822):928-33.
[41] Wang XL, McCredie RM, Wilcken DE. Common DNA polymorphisms at the lipoprotein lipase gene. Association with severity of coronary artery disease and diabetes. Circulation. 1996;93(7):1339-45.
[42] Mattu RK, Needham EW, Morgan R, Rees A, Hackshaw AK, Stocks J, et al. DNA variants at the LPL gene locus associate with angiographically defined severity of atherosclerosis and serum lipoprotein levels in a Welsh population. Arterioscler Thromb. 1994;14(7):1090-97.
[43] Jemaa R, Fumeron F, Poirier O, Lecerf L, Evans A, Arveiler D, et al. Lipoprotein lipase gene polymorphisms: Associations with myocardial infarction and lipoprotein levels, the ECTIM study. Etude Cas Temoin sur l'Infarctus du Myocarde. J Lipid Res. 1995;36(10):2141-46.
[44] Xie L, Li YM. Lipoprotein Lipase (LPL) Polymorphism and the risk of coronary artery disease: A meta-analysis. Int J Environ Res Public Health. 2017;14(1):84.

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