Research Article

Incidence of lipoprotein lipase D9N mutation and its association with risk of CAD and dyslipidemia in Indians

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Abstract

Introduction: LPL is one of the important enzyme for lipoprotein metabolism. Various mutations has been identified in different regions of LPL gene described so far, out of all D9N (Asp9Asn), Asn291ser and the Gly188Glu mutation seems to be most significant in CAD. This study was carried out to identify the incidence of Lipoprotein lipase D9N mutation and its association with risk of CAD and dyslipidemia in the western region of India. The study included total 160 case out of which 110 were CAD patients with 50 controls from 2010-13 referred to Department of Cardiology Dr. D.Y. Patil Hospital and Research Center, Nerul, Navi Mumbai. We have studied the three isoforms of LPL D9N mutations (DD, DN and NN) and correlated their association with dyslipidemia and CAD risk. **Conclusion:** Our study concluded that DD genotype is the most common genotype in both the groups: CAD and controls which shows a relatively lower risk of CAD. While DN and NN genotype has been found to be associated with a higher risk of CAD.
Keywords: LPL gene, CAD, dyslipidemia

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INTRODUCTION

Coronary artery disease (CAD) is the leading cause of death worldwide and is rapidly increasing in prevalence in developing countries[1]. CAD has been associated with behavioral, genetic and environmental risk factors[2,3]. Various comorbiditis like type 2 diabetes, hypertension, dyslipidaemia and obesity has been linked to CAD mainly via proinflammatory mediators[4,5]. India, with 2.9 million deaths in the age group 25-69, accounted for 25% of CAD deaths in developing countries in the year 1990 and this would likely to increase to 111% by 2020 as compared to 77% for china, 106% for other Asian countries and 15% for developed countries[6,7]. Prevalence of CAD in urban adults of India has increased fourfold in 40 years and even in rural areas it has doubled during this time[8]. The factors responsible for the increased prevalence of CAD in India include adoption of unhealthy lifestyles comprising lack of exercise and tobacco consumption, nutrition transition towards an atherogenic, cholesterol rich diet and socioeconomic transition associated with urbanization and industrialization[1]. The Indian subcontinent, with 1/6 of the world's population and with diverse ethnic, linguistic and cultural groups had always been the centre of focus for genetic studies. With evidence of the increasing prevalence of CAD during past two decades among Indians and Indian migrants in other countries, number of genetic association studies were conducted are very few. Lipid abnormalities play a significant causative role in the

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development of atherosclerosis and CAD. LPL hydrolyzes triglycerides in the core of both chylomicrons and Very Low Density Lipoproteins (VLDL), thus causing these particles to be transformed into chylomicron remnants and Intermediate Density Lipoproteins (IDL), Low Density Lipoproteins (LDL) respectively while surface molecules are transferred to the High Density Lipoprotein (HDL) fraction. Mutations in lipoprotein lipase may be implicated in causing increased blood Triglyceride with low HDL predisposing the individual to CAD[9]. However there are very few genetic studies with respect to Indian CAD patients. In the present study detection of the D9N mutations of LPL was carried out in CAD patients from Western region of India. The samples were analyzed with verified CAD for the presence of the D9N variant of lipoprotein lipase mutation. These genetic studies are helpful to examine the relative importance of genotypes compared with conventional cardiovascular risk factors on the total risk of CAD in our population at large.

AIMS AND OBJECTIVE

To identify the incidence of Lipoprotein lipase D9N mutation and its association with risk of CAD and dyslipidemia in Indians.

Lipoprotein lipase

Lipoprotein lipase (LPL) catalyses the hydrolysis of the triacylglycerol component of circulating chylomicrons and very low density lipoproteins, thereby providing non-esterified fatty acids and 2-monoacylglycerol for tissue utilization[10]. LPL are attached to the luminal surface of endothelial cells in capillaries by heparan sulfated proteoglycans. Genetically determined and metabolically induced disturbances in lipid metabolism, as manifested in several types of dyslipidemia, have been shown to be causally related to the development of CAD by increasing deposition of plagues in the coronary arteries leading to atherosclerosis[11]. Mutations in the LPL gene have been implicated in dyslipidaemia predisposing the individual to CAD[2,12]. The LPL gene is located on chromosome 8p22 and Spans ~30Kb. It is divided into 10 exons. The catalytic centre is formed by 3 amino acids Ser^{132}, Asp^{156}, and His^{241}. About 100 naturally occurring mutations in the LPL gene have been described. There are 61 missense mutations most of which are located in exons 5 and 6,12 nonsense mutations,[10] frame shift mutations,[3] gross mutations and splicing mutations[2]. Of the mutations described so far, the Asn291ser, D9N (Asp9Asn) and the Gly188Glu mutation seems to be most significant in CAD[13,14]. The D9N mutation causes a 20% decrease in LPL specific activity which causes increased triglyceride levels and low HDL level, leads to increased risk of CAD. The D9N increases triglycrides by 0.8 mmols and increased risk of CAD by 1.4 fold. The Asn 291 Ser mutation in LPL is a common mutation which causes slightly reduced enzymatic activity. It increases risk of CAD three fold in patients with familial hypercholesterolemia. The Gly188Glu mutation is frequent among French Canadians but it is widespread among population. It results in an enzymatically nonfunctional LPL. The Gly188Glu mutation causes chylomicronaemia and increases TG by80% and decreases HDL and increases risk of CAD by five fold [2].

Methodology

This study included 110 patients with CAD (stable angina, unstable angina, ST elevation Myocardial infarction and Non ST elevation MI) admitted in The Department of cardiology, Pad. Dr. D. Y. Patil Hospital and research center, Nerul, Navi Mumbai (Cardiac ICU and Ward) during period of 3 years and 50 control normal subject. All patients with CAD diagnosed on basis of clinical symptoms and sings, ECG and echocardiography underwent Coronary Angiography, lipid profile and Lipoprotein lipase D9N mutation study. All Control normal subjects (asymptomatic with normal ECG and normal Echocardiography) underwent lipid profile, Lipoprotein lipase D9N mutation study except coronary angiography. Written informed consent was obtained from each participant before inclusion in the study. The procedures used were in accordance with the guidelines of the Helsinki Declaration on human experimentation.

Coronary Angiogram

All patients with CAD except control patients underwent conventional coronary angiography by either femoral or radial route. Determination of CAD extent and severity coronary angiogram was done. Each angiogram was classified as revealing either lesion was on the left coronary artery (anterior inter-ventricular artery upper, middle and bottom segments and circumflex artery upper and bottom segments) or right coronary artery (upper and bottom segments).Lesion above 50 %, Proximal and Mid Segments were considered significant lesion. Disease in the distal segments was not considered because of difficulty in quantitation of the lesion severity in these areas.

Lipoprotein analysis

Triglyceride, Total cholesterol (TC), LDL cholesterol, HDL cholesterol and VLDL levels were measured by autoanalyser from 12 hours fasting venous blood samples.

LPL DNA analysis for the detection of Lipoprotien lipase D9N mutation

Fasting A 2-ml venous blood sample was drawn into an EDTA sample tube for the detection of LPL D9N mutation. DNA extraction was carried out by using Qi Amp Blood Mini Kit. Extracted DNA samples were checked for quality and quantity analysis. DNA was

amplified by PCR in a thermal cycler using specially designed oligonucleotide primers Restriction enzyme digestion of the amplification products was carried out. Finally separation of the digested products and identification of genotype was done by agarose gel electrophoresis[9].

Statistical Analysis

SPSS 16.0 statistical Package was used. To compare independent groups, one-way ANOVA was performed to compare continuous variables and Pearson Chi- square analysis was performed to compare categorical variables.

OBSERVATION AND RESULTS

Table 1: Demographic comparison between CAD and control groups

Variables	CAD (n=110)	Control (n= 50)
Age<45	27.3%	90.0%
Age >45	72.7%	10.0%
Male	76.4%	68.0%
Female	23.8%	32.0%
Hypertension	80.0%	.0%
Diabetes Mellitus	50.9%	.0%
Nicotine Addiction	71.8%	.0%
Alcoholic	13.6%	0.0%

In this study, 27% of the CAD patients and 90 % of the controls were below the age of 45 years and 72.7 % CAD cases and 10 % controls were above the age of 45 years. Gender wise (M/F) ratio for the present study is CAD (4:1) and control (3:1). In the CAD group, 80% patients were hypertensive, 50.9% were diabetic. It was observed that in CAD group, 71.8% subjects were consuming nicotine while 13.6% subjects were alcoholic.

Table 2: CAD and lipid profile parameters

Variables	CAD (110) Mean ± S.D.	Normal(50) Mean ± S.D.	'p' value
TC	209.95(±)29.50	166(±)34.68	.000
TAG	182.30(±)45.85	142.44(±)102.58	.000
VLDL	36.43(±)9.24	27.24(±)16.82	.000
LDL	135.43(±)25.79	92.8(±)25.79	.000
HDL	37.79(±)6.57	46.08(±)9.01	.000
NHDL-C	172.68(±)28.89	121.24(±)36.27	.000
TC/HDL	5.62(±)1.21	3.67(±)0.95	.006
LDL/HDL	3.66(±)0.99	2.07(±)0.66	.000

The present study observed a significant difference in lipid profile of CAD and normal controls. Circulatory levels of various parameters of lipid profile showed a significant variations as Total cholesterol(TC) (209.95±29.50/166±34.68), Triglycerides (182.30±45.85/142.44±102.58), Very low density lipoproteins (VLDL) (36.43±9.24/27.24±16.82), Low density lipoproteins (LDL) (135.43±25.79/92.8(±)25.79), High density lipoproteins (HDL) (37.79±6.57/46.08±9.01), Non HDL Cholesterol(NHDLC) (172.68±28.89/121.24±36.27), TC/HDL(5.62±1.21/3.67±0.95) and LDL/HDL (3.66±0.99/2.07± 0.66) between CAD and control group.

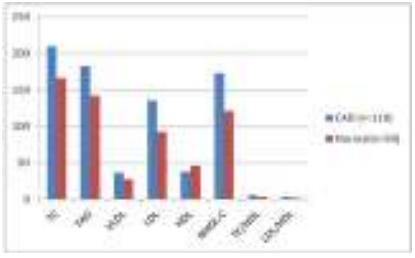
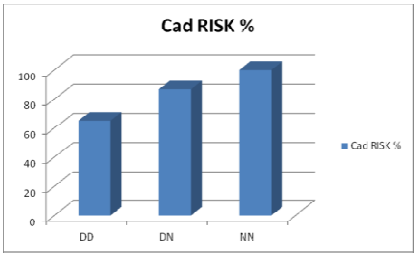


Figure 1: Lipid profile of CAD and control group

Table 3: Correlation of genotypes with CAD risk in control and cases

Variables (N)	DD	DN	NN
Cad (110)	90	13	7
Control (50)	48	2	0
Total (160)	138	15	7
Cad risk %	65.21	86.66	100



In the present study it was observed that DD genotype is the most frequent genotype found in both CAD and Control group which is associated with lower risk of CAD. While DN and NN genotypes seems to be associated with higher risk of CAD as compared to DD genotype (DN 86.66% and NN 100%).

DISCUSSION

This study was carried out to identify the incidence of Lipoprotein lipase D9N mutation and its association with risk of CAD and dyslipidemia in the western region of India. In our study we found that higher triglyceride , total cholesterol , LDL , VLDL with low HDL were associated with CAD as compare to controls[11,15]. We have studied the three isoforms of LPL D9N mutation and and reported that DD genotype is the most common genotype in both the groups: CAD and controls which shows a relatively lower risk of CAD. While DN and NN genotype has been found to be associated with a higher

risk of CAD. Similar study carried out by Fisher *et al* and Souverein *et al* showed that D9N mutation of LPL results in increased plasma triglyceride and decreased high density lipoprotein cholesterol concentrations with increased risk of genetic predisposition to atherosclerosis with increased risk of CAD[16,17]. Biological Mechanism behind D9N mutation leading to decreased enzyme activity and consequently elevated triglyceride levels and reduced HDL cholesterol levels should increase an individual's risk of ischemic heart disease. Elevated triglycerides indicate that IDLs, VLDLs and/or chylomicron remnants are present in plasma, and these particles may be selectively retained in the intima and consequently promote atherosclerosis. Reduced HDL cholesterol may result in reduced reverse cholesterol transport, indirectly promoting atherosclerosis[18,19].

CONCLUSION

Our study concludes that DD is a more frequent genotype found in both CAD patients and controls, associated with lower risk of CAD. While DN genotype seems to be associated with risk of CAD at a higher level compared to DD genotype. However the NN genotype showed the highest risk of CAD approximately, which can be considered as the most dangerous genotype. Various CAD risk factors have been identified by different studies with variable results. Very limited number of candidate gene studies has been conducted on Indian populations. Our study showed a high level of correlation of lipid profile and risk of CAD in specific genotype CAD patients (NN and DN). Further there is a need of extensive cohort studies in Indian population based on lipoprotein lipase mutations as it is directly associated with dyslipidemia and CAD risk.

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