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ORIGINAL ARTICLE

LIPOPROTEIN LIPASE GENE POLYMORPHISM – CORRELATION WITH SERUM TRIGLYCERIDES LEVELS

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ABSTRACT

Background and Objectives: Cholesterol (Total and LDL-cholesterol) level largely depends on lipoprotein metabolism. LPL gene rs328 (S447X) polymorphism is reported as gain of function by many investigators. Altered sequence of LPL mRNA showed less susceptibility to translation inhibition by adipocyte extract. Base change was found to be the possible cause of gain in function of LPL mRNA and resulted in lower susceptibility to inhibit-ion for translation. The aim of this study was to investigate the relationship of lipoprotein lipase (LPL) gene polymorphism with serum contents of triglycerides in the members of hyperlipidemia families.

Methodology: We selected twenty families having hyperlipidemia and coronary heart disease history from different regions of Punjab. Lipid levels of all the family members were determined. Individuals with or without hyperlipidemia were subjected to genotyping for LPL polymorphism (rs328). The allele-specific PCR was performed using high throughput fluorescent based genotyping Kaspar assay. Observed genotypes were confirmed by sequencing.

Results: Five families out of twenty were carrier of C>G transversion resulting in S474X change in LPL gene. Allele type and related lipid contents of each carrier family showed significant (p=0.04) increased levels of triglyceride in CC genotype compared to CG and GG genotypes. Similarly, HDL-cholesterol values were significantly raised (p=0.02) in CC genotypes than CG and GG.

Conclusion: LPL genotype S474X is a common variant in Pakistani hyperlipidemia families that might have a possible role in triglycerides and HDL-cholesterol regulation. Screening of mutation negative families for S474X alleles could be helping for cardiovascular disease risk assessment.

Keywords: Hyperlipidemia, HDL-cholesterol, genotype, S474 allele.

INTRODUCTION

Lipoprotein molecules are categorized into high density lipoproteins, intermediate density lipoprotein, low density lipoproteins and very low density lipoproteins. The high density lipoprotein molecules are very low in cholesterol contents and are known as good lipids while low density lipoproteins have high cholesterol contents and known as bad lipids. When level of low density lipoprotein increases in the systemic circulation it enhances the atherogenesis. Cholesterol (Total and LDL-cholesterol) level largely depends on lipoprotein metabolism.

Lipoprotein lipase (LPL) is a glycoprotein mainly synthesized in the adipose and muscle cells which catalyzes the hydrolysis of triacylglycerol in chylomicrons and very low density lipoprotein. It serves the triglyceride lipase activity. It is secreted and transported to the vascular endothelium surface and bound to heparin sulfate proteoglycans. It also expresses in macrophages derived foam cells in atherosclerotic sites.[1,2] Serum LPL familial deficiency has incredible high plasma triglycerides and lower very low density lipoprotein (VLDL). Protective effect of S447X allele for coronary artery disease was studied on scrutinized 178 patients after coronary angiography, clinical examination and diagnosis. Genotyping results for S447X allele showed prevalence of frequency higher in non-CAD participant than in CAD patients.[3] Genetic variants in LPL gene and their relationship with lipid contents and obesity in Chinese children showed an increased risk of obesity. Similarly, in addition to LPL gene variants, higher triglyceride level showed association with higher (1.32 fold) risk of obesity. While increased high density lipoprotein cholesterol level was found to be associated with 36% lower risk of obesity.[4] LPL gene rs328 (S447X) polymorphism is reported as gain of function by many investigators. Altered sequence of LPL mRNA showed less susceptibility to translation inhibition by adipocyte extract. Base change was found to be the possible cause of gain in function of LPL mRNA and resulted in lower susceptibility to inhibition for translation.[5] The aim of this study was to inves-

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tigate the relationship of lipoprotein lipase (LPL) gene polymorphism with serum contents of triglycerides and high-density lipoprotein (HDL) cholesterol among hyperlipidemia families.

MATERIALS AND METHODS

Detailed family history and written informed consent of each participant was taken for this study. We selected twenty mutation negative families having hyperlipidemia and coronary heart disease history from different regions of Punjab. DNA was extracted with modifications in the standard organic method from leukocytes. Pedigrees were drawn to see the genetic inheritance pattern of each family.

Serum Lipid Profiling

Lipid levels of all the family members were determined using spectrophotometry method.

Genotype

Individuals with or without hyperlipidemia were genotyping for *LPL* polymorphism (rs328). Sequence used to locate rs328 SNP is as 5'TGAATAAGAAGT3'. Amplification conditions were used described and allele-specific PCR was performed using high through put fluorescent based genotyping Kaspar assay following the already published protocol.[6] Sanger sequencing of the selected genotyped samples was done for confirmation.

Data Analysis

Lipid profile data was analyzed using t-test and sequence data was analyzed using BLAST2 tool. Sequence detection system software was used to analyze the data

RESULTS

Five families out of twenty were carrier of C > G transversion resulting in S474X change in LPL gene.

Allele type and related lipid contents of each carrier family showed significant (p = 0.04) increased levels of triglyceride in CC genotype compared to CG and GG genotypes. Similarly, HDL- cholesterol values were significantly raised (p = 0.02) in CC genotypes than CG and GG.

DISCUSSION

We have genotyped S447X allele to estimate the prevalence in the FH families. Five families out of twenty were carrier of C>G transversion resulting in S474X change in LPL gene. Allele type and related lipid contents of each carrier family showed significant (p = 0.04) increased levels of triglyceride in CC genotype compared to CG and GG genotypes.

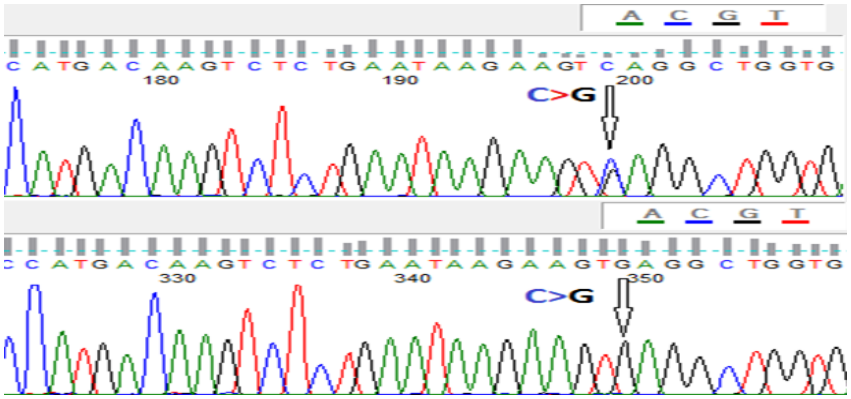

Fig. 1: *Sequencing result of rs328 polymorphism.*

Table 1: *Serumlipid level in the study individuals.*

	C allele carrier		*G allele carrier*		*p-value*
	Mean	*St. Dev.*	*Mean*	*St. Dev.*	
Age (years)	39.7	±3.5	40.7	±4.9	0.07
TC (mmol/L)	6.81	±1.5	4.92	±0.5	< 0.05
Tri-gly (mmol/L)	7.16	±1.9	6.03	±1.8	0.046
HDL-C (mmol/L)	1.12	±0.1	1.28	±0.3	0.024
LDL-C (mmol/L)	4.38	±1.8	2.32	±0.6	< 0.05

A large group of publications have depicted the significance of triglyceride levels and the underlying consequences of cardiac problems. Lipoprotein lipase gene has impact on triglyceride levels and more commonly few polymorphisms are evaluated for association with triglycerides.

Higher serum levels of triglycerides were observed genotype in comparison south Indians.[7] Metabolic syndrome in Koreans were investigated[8] and haplotypes of lipoprotein lipase gene for Pvu II and Hind III alleles were constructed. In total 269 Met Syn patients were evaluated for their genotype, LPL mass, high density lipoprotein lipase, triglycerides, waist circumference, blood pressure and their insulin metabolic values. Mutant allele of Pvu II and Hind III had lower LPL mass in patients. The odd ratio for carrier of both mutant alleles also showed higher values in high carbohydrate intake cohort. Susceptibility for cardiovascular risk factors like Hypertension and type II diabetes association with polymorphisms in lipoprotein lipase gene were evaluated [9] in Mexico families. Two common variants S447X and Hind III were genotyped

for cardiovascular risk relationship in 30 families. Hind III T/T genotype found to be associated with raised diastolic blood pressure. Association of risk of lipodystrophy and dyslipidemia development in HIV patients was investigated[10] in 174 patients. Ploymorphisms in APOE and LPL gene were also screened to determine the risks. Genotypes with S447X were observed with decreased values of cholesterol (OR 0.39 and p = 0.05). Frequency of LPL gene rare variants was determined[11] in 313 patients with hypertriglyceridemia and type III hyperlipidemia. The LPL gene sequenced for coding and intron-exon boundaries. In total 20 variants were identified of which 7 were already reported. Sixteen missense variants with two short deletions, three nonsense and one insertion variant were found. Carriers of rare variants were found to be elevated level of triglycerides than the non-carriers. Similarly, HDL-cholesterol values were significantly raised (p = 0.02) in CC genotypes than CG and GG.

It is ***concluded*** that *LPL* genotype S474X is a common variant in Pakistani hyperlipidemia families that might have a possible role in triglycerides and cholesterol regulation. Screening of families for S474X alleles could be helpful for risk assessment of cardiovascular disease.

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Author's Contribution

HJS: Study design. HB: Sample collection. MUS: Sample collection and writing. MYB: Sample collection and writing. AA: Study design, data analysis and manuscript writing.

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