Annals of Biology **38** (1) : 145-149, 2022

Impact Score : 0.32 (Scopus)

Relevance of LDL Genetic Polymorphism as Cardiovascular Diseases Risk Estimates

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(Received : December 20, 2021; Accepted : February 11, 2022)

ABSTRACT

Cardiovascular disease is very common worldwide. Low density lipoprotein (LDL) is one of the most important factors which plays an important role in cardiovascular disease. The aim of this study was to evaluate frequency distribution of LDL gene SNPs in CVD patients to find out any association with risk of disease. The present study was carried out on blood samples collected from CVD suspected patients to study risk of disease. The AA (wild type) and AB heterozygote genotype frequency were lower in cardiovascular cases. In case of familial hypercholesterolemia (FH) percentage, frequency was higher in heterozygous groups. Percentage frequency of wild type (AA) group was found lower. Allele frequency of B allele was found more prevalent in patients affected with cardiovascular disease. Higher values of total cholesterol, serum triglycerides, HDL, LDL and VLDL showed association with higher prevalence of CVD risk factors. The present results suggested the difference of genotype distribution of LDL polymorphism between cardiovascular patients; hence, established the association with risk of disease. Further validation studies are required on additional LDL SNPs to study the CVD risk using larger cohort.

Key words : Cardiovascular disease, LDL, genotype, single nucleotide polymorphism (SNPs), lipid profile

INTRODUCTION

Heart disease is one of the leading causes of death worldwide. Heart disease risk may be handed down from generation to generation through genes. Heart failure is a major clinical problem, therefore, methods to protect heart against the changes which lead to failure in heart such as pathological cardiac hypertrophy is becoming more and more critical. Protein-coding genes are most well studied sequences but account for approximately < 2% of the genome only. Previously unknown non-coding RNA species (formerly known as junk) have been discovered, and added a new dimension and complexity to DNA, RNA and protein regulation.

According to World Health Organization (WHO), Department of Non-communicable Diseases and Mental Health has done commendable work on major non-communicable diseases, like diabetes, cancer, asthma, cardiovascular disease and some mental illnesses. To discover genes for cardiovascular disease (CVD) and its risk factors in humans, two major approaches – genetic association and linkage analysis have been utilized. Some studies have found CVD showing a simple pattern of inheritance, which suggested single causal gene which has large phenotypic effect (Menon *et al.*, 2014). Single nucleotide polymorphisms known as SNPs are the most common type of genetic variation in people. SNP represents a difference in a single DNA base, known as nucleotide.

The LDLR gene provides information to make a protein called a low-density lipoprotein receptor (Buraczynska *et al.*, 2021; Miksenas *et al.*, 2021; Trinder *et al.*, 2021). This receptor binds to low-density lipoproteins (LDLs), which are the primary (1^0) carriers of cholesterol in the blood. Hence, this study was designed to identify polymorphism in the genomic sequence of LDLR gene and to associate genetic variants of LDLR with CVD.

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The present study aimed at evaluating frequency distribution of LDL gene SNPs (Ava II) existing in exon 13, in cardiovascular patients to find out any association with risk of disease. Serum cholesterols, triglycerides and HDL level were also examined in order to determine their diagnostic and prognostic values. Samples collected were categorized into two groups : healthy and diseased samples. The basis of categories was serum cholesterol, serum triglyceride, VLDL, HDL and LDL levels.

MATERIALS AND METHODS

The present study was carried out on blood samples collected from CVD patients. The samples were collected from various hospitals such as Healing Touch Hospitals, Max Hospital and Dr. Lal Path Labs.

The DNA was extracted from peripheral blood by using phenol chloroform extraction method. The purity was checked spectrophotometrically. Quality check was done by agarose gel electrophoresis. Purity of DNA sample was checked by OD at OD260/OD280. The DNA prepared from blood was 20-50 Kb in size and suitable for use as a template in PCRs.

Genotyping of LDL gene polymorphisms was done by amplification of genomic regions containing the polymorphisms with set of primers designed by Primer 3-software (Table 1). Primers were purchased from Sigma aldrich. The sequence selected was Exon 13 LDL gene. Ava II enzyme was used to detect polymorphism. PCR amplification was performed by a bio rad thermocylcer. PCR mixture is shown in Table 2.

Amplification process was done on denaturation at temperature 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, annealing for 30 sec and 72°C for 30 sec, and a final extension at 72°C for 5 min. The final PCR product contained intron 13 and exon 13 of the LDL gene. The PCR product was subjected to restriction endonuclease digestion Ava II restriction enzymes.

Table 2. PCR protocol (25 µl reaction mix)

Constituents	Quantity (µl)
Genomic DNA (100 ng/µl)	2.0
PCR Master mix (Fermentas)	12.5
Milli Q water	9.5
Forward primer	0.5
Reverse primer	0.5

Results are expressed in number and percentage.

Genotypic frequency = (No. of samples with specific genotypes)/(Total number of samples) Gene frequencies : A = AA + ½ AB

B = BB + ½ AB

Where,

A, B=Gene frequencies

AA, BB=Genotype frequency of homozygote

AB=Genotype frequency of heterozygote

RESULTS AND DISCUSSION

The present study provides preliminary information regarding association of genetic variants of LDL gene with CVD. In this study, complete exon 13 LDL genes to partial intron 14 and partial intron was targeted of LDL gene. PCR-RFLP was performed for LDL gene. Ava II enzyme was used to detect polymorphism (RFLP). The PCR product of 257 bp size of LDL gene was digested with Ava II restriction enzyme. Three types of genotype were observed i.e. AA, AB and BB (Fig. 1). The presence of two bands indicated heterozygous trait which meant both alleles were present. Polymorphism was found in LDL gene affected population.

In the present study, LDL gene polymorphism was examined in cardiovascular patients (Table 3). In SNP Ava II, maximum cardiovascular patients were found in mutant condition (BB). The AA (wild type) and AB heterozygote genotype frequency were lower in cardiovascular cases. In case of familial hypercholesterolemia (FH) percentage, frequency was higher in heterozygous groups (Table 4). Percentage frequency of wild type (AA) group was found lower. Allele frequency was counted in cardiovascular patients for LDL

Table 1. PCR oligonucleotide primers of LDL gene

Primer		Sequence (5′→3′)	Annealing temp. (°C)	Amplicon size (bp)
Primer 1	FR	TTCCTTGCTGCCTGTTTAGG		
		TCAGCTATACCAGAAGATTCCAGA	58.3	256

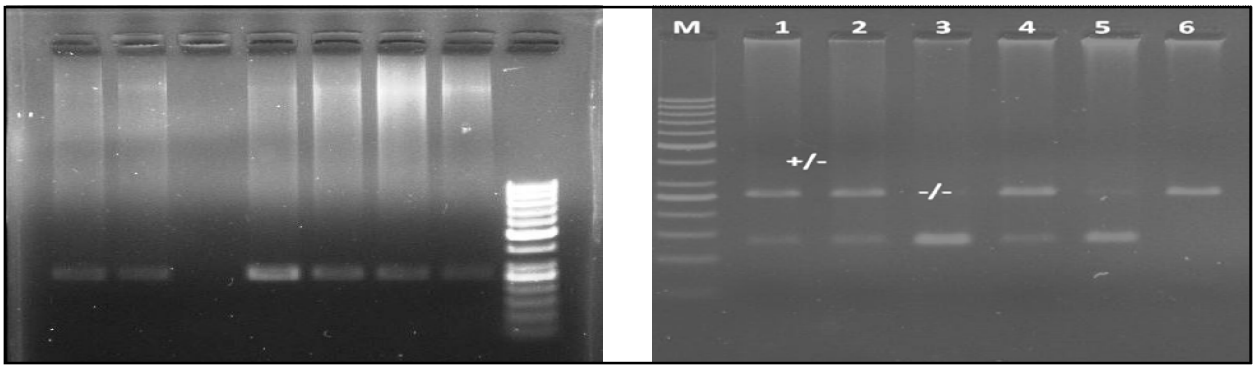

Fig. 1. Representative results of PCR-RFLP of LDL gene. (a) Lane 1-7 : PCR product (257 bp) and M : 50 bp DNA ladder and (b) PCR-RFLP (Ava II) Primer I LDLR gene.

Table 3. Genotype and allele frequency of cardiovascular patients (CVD)

Genotype (%)	AA	AB	BB	A	B
Genotypic frequency	25	25	50	37.5	62.5

Table 4. Comparison of genotype distribution and relative allele frequencies of polymorphisms of the LDL with heterozygous Familial Hypercholesterolemia (FH)

Genotype (%)	AA	AB	BB	A	B
Genotypic frequency	16	50	34	41	59

gene polymorphism. B allele was found more prevalent in patients affected with cardiovascular disease (Table 3).

Cholesterol levels were found high in diseased samples as compared to healthy serum samples. Diseased female showed more serum cholesterol as compared to healthy female. Comparison between male and female serum sample concluded significant increased level of serum cholesterol in diseased female as compared to diseased male (Table 5).

Average serum triglyceride levels were calculated. Healthy male and female serum sample showed lower values as compared to their diseased sample, respectively. Female serum triglycerides were lesser as compared to male diseased serum sample. No statistical significant difference was observed among female and male triglyceride.

HDL levels were calculated from biochemical analyzer, which showed higher HDL levels in diseased samples of both categories i.e. male and female. There was no significant difference of HDL level when observed among healthy male and female serum samples. HDL levels were lower in diseased female as compared to diseased male serum samples. LDL levels showed high levels in diseased serum samples as compared to healthy one. Maximum level of LDL was found in diseased female serum samples. Average level of VLDL obtained from serum sample of female and male population showed that maximum level was in diseased female serum sample. Comparison of healthy male and diseased male serum sample concluded that level of VLDL was high in diseased both in male and female population (Table 5).

In the present study, association of LDL gene polymorphism and lipid profile was studied in relation with cardiovascular disease. The present study provided the information about higher total cholesterol, serum triglycerides, LDL, HDL and VLDL. The important findings of the present study showed that patients with mutant genotype (BB) of LDL gene and heterozygote (AB) of familial hypercholesterolemia were associated with cardiovascular disease. Higher values of total cholesterol, serum triglycerides, HDL, LDL and VLDL showed

Table 5. Average of serum cholesterols, serum triglycerides and HDL in healthy and diseased male and female samples

Characteristics	Normal (Male)	Diseased (Male)	Normal (Female)	Diseased (Female)	Normal values
Total cholesterol	169.75	242.33	140.0	266.66	~148
Serum triglycerides (mg/dl)	169.8	248.7	144.5	378.4	~115
HDL (mg/dl)	47.1	80.0	48.1	71.2	~56
LDL (mg/dl)	98.7	147.0	122.0	163.8	~62
VLDL (mg/dl)	25.5	45.0	30.0	73.67	~23

association with higher prevalence of CVD risk factors.

The prognostic and therapeutic implication of LDL gene polymorphism in cardiovascular patients was recognized (Piko *et al.*, 2017). LDL gene polymorphism status is an important predictive and prognostic factor in cardiovascular disease. Many studies found significant association of LDL gene polymorphism with cardiovascular disease similar to present results (Bahadir *et al.*, 2015; El-Lebedy *et al.*, 2016). Few studies found no statistically significant association between LDL polymorphism and risk of cardiovascular disease (He and Wang, 2015; Franczak *et al.*, 2018).

Paquette *et al.* (2017) indicated that familial hypercholesterolemia (FH) is a monogenic disease; it has been shown that genetic variants and clinical risk factors can modify cardiovascular disease (CVD) risk similar to our results (Paquette *et al.*, 2017). Yang (2015) had also reported that lipoprotein index could act as a good predictor for CVD.

Still there is continuing debate over utility of lipids for CVD risk prediction. The magnitude of total cholesterol and high density lipoprotein was maximum ion CVD diagnosis and assumption (Welsh *et al.*, 2019). Another recent trend setter study showed that apolipoproteins had improved CVD risk measurement of total cholesterol and HDL-C (Du *et al.*, 2014).

Previous studies reported importance of the lipoprotein index, such as LDL/HDL ratio, to identify healthy subjects and patients in a large population (Giudicessi *et al.*, 2017). Previous studies showed that the LDL/HDL ratio was useful in finding the risk of early stage atherosclerosis in Japanese type 2 diabetic patients. The present study showed that the higher HDL, LDL and VLDL values were significantly correlated with high prevalence of CVD risk factors similar to other results.

Low-density lipoprotein (LDL) is a well-established risk factor for CVD. Reduced LDL has been a cornerstone in the treatment and prevention of atherosclerosis. Intensive statin therapy has been shown to significantly reduce the occurrence and mortality of cardiovascular disease (CVD).

There are residual cardiovascular risk still occurs in few patients despite of achieving of LDL goals (like polymorphism and lipid profile). The residual cardiovascular risk is complex, partly due to atherogenic dyslipidemia, which is characterized by lower levels of high-density lipoprotein and elevated plasma triglyceride concentrations.

CONCLUSION

In the present study, population genetics was studied which had come out with finding that LDL gene polymorphism and higher levels of lipid profile were present in Chandigarh population. Allele B was most abundantly present in the targeted population. Chances of inheritance of CVD were also high in the targeted population. Higher values of total cholesterol, serum triglycerides, HDL, LDL and VLDL showed association with greater prevalence of CVD risk factors. Improvement in health, lifestyle and regular work out is the demand of healthy heart in Chandigarh. Regular health checkup system must be updated and health awareness should be more in the region.

ACKNOWLEDGEMENT

The authors are grateful to the Chandigarh Group of Colleges for providing the help and support.

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