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Genetic spectrum of low density lipoprotein receptor gene variations in South Indian population

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A R T I C L E I N F O

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A B S T R A C T

Background: Low density lipoprotein receptor (*LDLR*) is a membrane bound receptor maintaining cholesterol homeostasis along with Apolipoprotein B (*APOB*), Proprotein Convertase Subtilisin/Kexin type 9 (*PCSK9*) and other genes of lipid metabolism. Any pathogenic variation in these genes alters the function of the receptor and leads to Familial Hypercholesterolemia (FH) and other cardiovascular diseases.
Objective: This study was aimed at screening the *LDLR, APOB and PCSK9* genes in Hypercholesterolemic patients to define the genetic spectrum of FH in Indian population.
Methods: Familial Hypercholesterolemia patients (n = 78) of South Indian Tamil population with LDL cholesterol and Total cholesterol levels above 4.9 mmol/l and 7.5 mmol/l with family history of Myocardial infarction were involved. DNA was isolated by organic extraction method from blood samples and *LDLR*, *APOB* and *PCSK9* gene exons were amplified using primers that cover exon-intron boundaries. The amplicons were screened using High Resolution Melt (HRM) Analysis and the screened samples were sequenced after purification.
Results: This study reports 20 variations in South Indian population for the first time. In this set of variations 9 are novel variations which are reported for the first time, 11 were reported in other studies also. The *in silico* analysis for all the variations detected in this study were done to predict the probabilistic effect in pathogenicity of FH.
Conclusion: This study adds 9 novel variations and 11 recurrent variations to the spectrum of *LDLR* gene mutations in Indian population. All these variations are reported for the first time in Indian population. This spectrum of variations was different from the variations of previous Indian reports.

1. Introduction

LDLR gene spans around 45 kb with 18 exons and codes for the low density lipoprotein receptor (LDLR) with 860 amino acids [1]. This receptor manages to deliver LDL particles from the blood stream to liver for recycle of lipids and lipoproteins. The Apolipoprotein B (*APOB*) binds to LDL particle and helps in recognition of LDL particle by the LDL receptor [2]. Proprotein convertase subtilisin/kexin type 9 (*PCSK9*) is responsible for the recycling of LDL receptor for the next cycle [3–6]. *LDLR* gene has been extensively studied by various investigators to discover the recurrent and unknown mutations in different population. The mutations in this gene lead to Familial Hypercholesterolemia (FH), a dominant autosomal disorder characterized by elevated plasma cholesterol level which in turn causes cardiovascular disease [7]. The pathogenic mutations in *APOB* [8] and *PCSK9* genes are also known to contribute to the FH condition, but, apart from these genes, there are recent reports supported by genome wide association studies, claiming that certain single nucleotide polymorphisms (SNPs) in the genes of lipid metabolism also have an additional effect to the condition suggesting that FH is rather a polygenic disorder than monogenic. Thus, it is evident that the SNPs in these genes could play an indispensable role in determining the genetic risk factors that cause FH, cerebrovascular disease [9] and cardiovascular disease [10]. Haiyan Zhu et al., reported that the common polymorphism rs688 alters the exon splicing efficiency and is associated with increased cholesterol level in Caucasian population [11]. Feng Gao in 2012, using *in vitro* studies demonstrated the molecular mechanism of the receptor due to this polymorphism [12]. Miljkovic et al., reported that rs5929 SNP is associated with increased LDL cholesterol levels in African population [13]. A number of other polymorphisms in *LDLR* gene (rs14158,

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Table 1
PCR – HRM analysis condition.

S. no	Component	Amount/reaction
1	Resomix (Roche)	10 µl (2 ×)
2	Genomic DNA	10 ng
3	Forward primer	10 picomoles/µl
4	Reverse primer	10 picomoles/µl

Annealing T (°C)	Fragments included	HRM temp (°C)
62	3, 4(2),5,6, 7, 10(1), 11, 13, 17	80–95
57	2, 12, 14, 15, 18, *APOB*, *PCSK9*	80–95
67	Promoter, 1, 4(2), 4(3), 8, 9, 10 (2), 16	80–95
Separate PCR	4(4)	80–95

Table 2
Baseline characters of the patients involved in study.

Parameters	Possible FH (n = 78)
Age	48.99 ± 14.34
Gender (m/f)	46/32
BMI (kg/m^2)	25.6 ± 3
Total cholesterol (mmol/l)	7.52 ± 1.71
Triglycerides (mmol/l)	3.29 ± 3.04
LDL-C (mmol/l)	5.06 ± 1.78
HDL-C (mmol/l)	1.21 ± 0.46
Xanthoma	Not observed
Corneal arcus	Not observed
Family history of MI	78
Smoking	42
Diabetes	38

rs3826810, rs1433099, rs2738464, rs2738465, and rs2738466) has been associated with cardiovascular diseases. A meta-analysis report showed that the SNP rs2228671 of *LDLR* is associated with Coronary Heart Diseases risk in European population but not in Chinese population [14]. This report considers the common polymorphisms along with other variations for analyzing their pathogenicity in causing cholesterol associated diseases.

The exon-intron junctions of *LDLR* have splice site donor and acceptor sites which are flanked by exonic splice enhancers (ESE) that recruit SR proteins that are essential for the formation of spliceosome complex [15–18]. The intronic variations may modify the random cryptic splice site to a higher affinity site than the authentic site which results in altered splicing and in turn a truncated protein. F. Takeuchi in 2012 has shown that the intronic variations led to altered splicing and are related to an increase of LDL cholesterol levels and are risk factors for Cardiovascular Diseases [19].

There are extensive reports available on various population all over the world, but Indian population has not been explored widely. There are very few reports available on Indian population [20,21,22]. Therefore, methodical population screening is imperative in the Indian subcontinent due to its diverged population and inbred communities to identify the risk alleles and other factors causing the disease. Based on UCL *LDLR* database [23] and Universal Mutation Database [24–31], the gene has > 1200 variants throughout the world, and only 38 has been reported in Indian population which is very trivial for such a diverged population. Further, reports suggest that India has greater risk of cardiovascular disease due to the changing food and life style [32] so it becomes indispensable to analyze and document the variations in *LDLR*, *APOB* and *PCSK9* genes in the population.

Our study was primarily focused on identifying pathogenic variations of *LDLR*, *APOB* and *PCSK9* genes in the Indian population. The recurrent, novel variations and common polymorphisms were documented in this report and also the intronic variations were given equal importance while verifying the variations. The variations obtained in *LDLR* gene in this population, were analyzed by *in silico* tools and by meta-analysis. *In silico* analysis of the variations (intronic and exonic) serves as a better approach to predict the pathogenicity of the variations, hence, the observed polymorphisms, mutations and intronic variations were scrutinized using appropriate tools and the results were equated with the previous reports from various databases and research articles.

2. Materials and methods

2.1. Subject recruitment and sample collection

The patients enrolled at SRM Medical College Hospital and Research Centre, Chennai, India for Master health check-up, which includes cholesterol and lipid profile examination were considered for the study. The cholesterol profile, history of CVD and clinical symptoms of FH were evaluated by clinicians and biochemists at the Biochemistry testing laboratory. There were 78 patients involved in this study, with elevated total and LDL cholesterol levels according to UK–Simon Broome criteria (TC > 7.5 mmol/l and LDLC > 4.9 mmol/l) with family history of Myocardial Infarction (MI), were considered for the study. After obtaining the written consent from the patients, 2 mL of peripheral blood sample or blood clot (remaining part after cholesterol estimation) was taken for further DNA based molecular analysis. The study was approved by SRM University Institutional Ethical Committee (IEC/2013/488).

2.2. DNA extraction and PCR-HRM analysis

DNA was isolated from the whole blood sample by phenol-

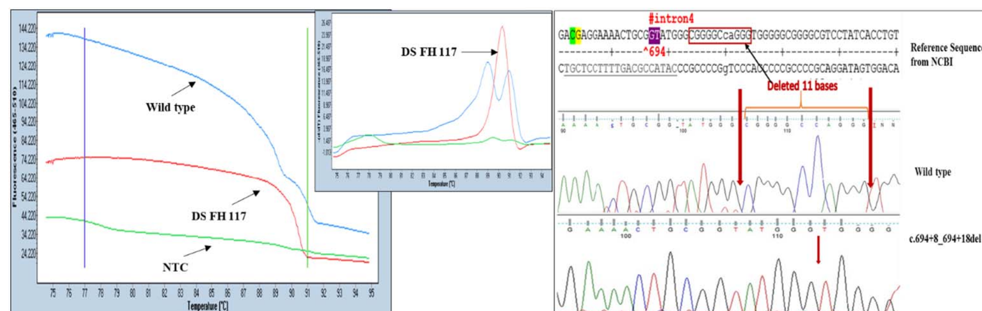

Fig. 1. Sequencing result of DS FH 117 showing c.694 + 8_c.694 + 18del homozygous condition.

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