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Case Report

Genetics Section

Variant Analysis in LDLR Gene Uncovers Genetic Basis of Familial Hypercholesterolemia: A Case Report

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ABSTRACT

Familial Hypercholesterolemia (FH) is a hereditary disorder characterised by elevated blood cholesterol levels, predominantly Low-density Lipoprotein Cholesterol (LDL-C). This condition poses a significant risk for early-onset atherosclerotic cardiovascular diseases. A critical step toward effective clinical management is the precise identification of pathogenic variants responsible for FH. The present study aimed to unravel the genetic cause of FH through comprehensive variant effect prediction and comparison with clinical manifestations in a nine-year-old girl with hyperlipidemia. Whole Exome Sequencing (WES) was performed on the proband, and a set of three key genes associated with hyperlipidemia {Apolipoprotein E (APOE), Low-density Lipoprotein Receptors (LDLR), Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9)} were evaluated for the presence of pathogenic mutations. The data were meticulously analysed based on the American College of Medical Genetics (ACMG) guidelines for variant classification. The analysis revealed two pathogenic variations in the LDLR gene: c.1A>C (p.Met1Leu) in exon 1 and a splice site variant c.1187-10G>A in intron 8. Sanger sequencing of family members confirmed the presence of one mutation each in the father and mother, while a younger sibling also carried both pathogenic variants. Genetic testing confirmed Heterozygous FH (HeFH) in the parents and Homozygous FH (HoFH) in both siblings. Proper classification of genetic variants is crucial for informed clinical decision-making and patient management. The study provides valuable insights into the molecular basis of FH in an Indian patient and contributes to the growing knowledge of the LDLR gene mutation spectrum.

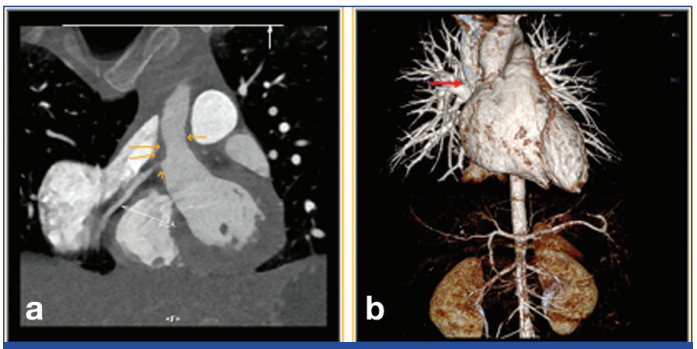
Keywords: Coronary heart disease, Gene mutation, Low-density lipoprotein receptor, Whole exome sequencing

CASE REPORT

A nine-year-old female child presented to the Cardiology Department of the hospital with chief complaints of fatigue, malaise, breathlessness, and chest discomfort for a month. Medical history revealed that she had experienced severe dyspnoea while climbing stairs a month ago, which prompted further evaluation. She had a coronary event, and her lipid profile revealed hyperlipidemia. Family history revealed that her parents and younger sibling also had hyperlipidemia. Her Total Cholesterol (TC) was 691 mg/dL, and Low-density Lipoprotein Cholesterol (LDL-C) was 650 mg/dL. Statin therapy was initiated for the patient, and within a month of this episode, she was referred to the Cardiology Department of a super specialty centre for an in-depth assessment and management.

Radiological Investigations

Cardiac Computed Tomography (CT) Angiography showed diffuse hypo-enhancement of the subendocardial portion of the interventricular septum, and the lateral and apical walls of the left ventricle. There was mild supravalvular aortic stenosis with mild aortic wall thickening and calcification. Mild wall thickening and soft wall plaques were noted in the descending thoracic and abdominal aorta. The coronary angiogram showed 80% ostial stenosis before bifurcation, 90% block in the proximal Left Anterior Descending artery (LAD), and 90% ostial tight lesion in the right coronary [Table/Fig-1a,b]. Therefore, a final diagnosis of critical coronary artery disease involving all three coronary arteries was established. Based on these findings, Coronary Artery Bypass Graft surgery (CABG) was performed with total arterial revascularisation employing an off-pump technique. Bilateral internal mammary arteries were employed for coronary artery grafting. Postoperatively, the patient was successfully managed with oral therapy and discharged.



[Table/Fig-1]: a) Coronary CT Angiogram showing severe Right Coronary Artery (RCA) ostial stenosis, supravalvar aortic wall thickening; and b) Volume rendered image showing supravalvar aortic stenosis (red arrow).

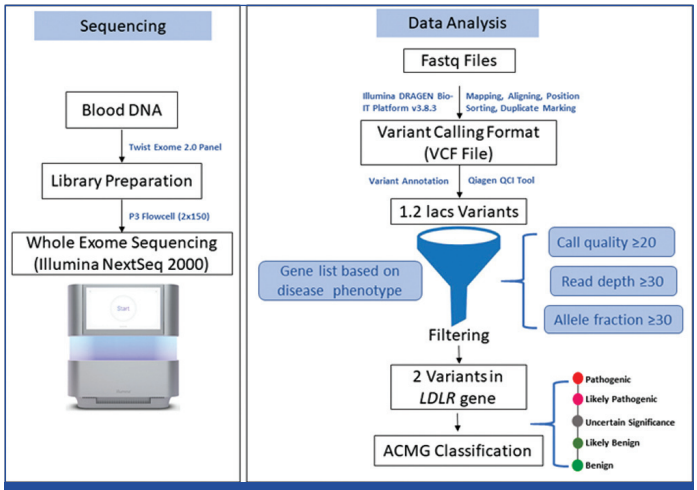
Genetic Work-up

The case was then referred to the genomics division of the hospital for genetic investigations. Subsequently, molecular testing was performed for both the parents and younger sibling. Informed consent was obtained from all individual participants included in the study, while the parents consented on behalf of the index patient. Whole Exome Sequencing (WES) was performed on the proband to identify the potential genetic defect associated with the phenotype. Libraries were prepared using the Twist Exome 2.0 Panel (Twist Bioscience, San Francisco, CA, USA), and Next Generation Sequencing (NGS) was performed on the NextSeq 2000 platform (Illumina, San Diego CA, USA).

Sequencing Data Analysis

The Deoxyribonucleic Acid (DNA) sequence was mapped to the published human genome build UCSC hg38/GRCh38 reference, and the Variant Calling File (VCF) was created using the Illumina DRAGEN Bio-IT Platform v3.8.3. The VCF file was uploaded to

the QIAGEN Clinical Insight Interpret tool (QCII, QIAGEN, Hilden, Germany), and the variants were filtered based on call quality, read depth, allele fraction, and phenotype/FH-related key genes (LDLR, APOB, PCSK9) [Table/Fig-2]. A total of 1.3 lakh variants were obtained. Filtration and prioritisation of variants based on phenotype showed that the proband carried two variants in the LDLR gene; NM_000527.5: c.1A>C (p.Met1Leu) in exon 1 and a splice site variant NM_000527.5:c.1187-10G>A in intron 8. The [Table/Fig-3] shows Integrative Genomics Viewer (IGV) visualisation of both LDLR variants: c.1A>C (p.Met1Leu) and c.1187-10G>A. Both these variants were further characterised to establish their pathogenic nature.



[Table/Fig-2]: NGS-based Genetic Analysis Workflow: WES was performed and NGS was aligned to a reference genome, followed by alignment sorting, duplicate removal, variant calling, annotation and filtering. Variants in the genes (APOE, LDLR and PCSK9) associated with FH were further classified based on ACMG guidelines.
NGS: Next generation sequencing

[Table/Fig-3]: Integrative Genomics Viewer (IGV) visualisation of LDLR variants: c.1A>C (p.Met 1 Leu) and c.1187-10G>A.

Classification of variants: The pathogenicity of the germline variants obtained was determined based on the American College of Medical Genetics (ACMG) variant classification guidelines [1] using clinical and population databases such as ClinVar, Human Gene Mutation Database (HGMD), genome Aggregation Database (gnomAD) and gnomAD. In-silico analysis tools such as PolyPhen, MutationTaster, Combined Annotation Dependent Depletion (CADD), Rare Exome Variant Ensemble Learner (REVEL) SpliceAI-10k, and MaxEntScan were used to support variant classification.

The variation c.1A>C (dbSNP: rs879254382), a loss of function null variant, results in the disruption of the initiation codon methionine. It has been reported as pathogenic by reputable sources such as ClinVar (VCV000250966.6- reviewed by an expert panel) and HGMD and is absent from controls according to the gnomAD database. The variant is detected in trans (as confirmed in this study by parental investigations) with another disease-causing splice site variant (c.1187-10G>A) in the LDLR gene. The in-silico analysis using Polyphen and Mutation Taster indicates deleterious or disease-causing effects and higher scores on CADD (21.2) and REVEL (0.638) analysis. The proband's phenotype is highly specific for a disease caused by the LDLR gene.

The other variant, c.1187-10G>A, activates a cryptic splice site that results in intron inclusion, as confirmed by wet-lab and bioinformatics analyses [2]. It has been reported in the ClinVar and HGMD databases as a pathogenic variant. Multiple lines of computational evidence support a deleterious effect on the gene or gene product (CADD=23.9, MaxEntScan, SpliceAI). Co-segregation of this variant with FH phenotype has been observed in multiple affected family members in a study by Sun LY et al., [3]. The prevalence of the variant in the population is extremely low (0.0022% gnomAD) and it has been detected in trans with another disease-causing variant in the LDLR gene in a case of homozygous FH [4].

Based on this evidence, both variants were classified as class 5 pathogenic variants. Targeted LDLR mutation analysis by Sanger sequencing in the family members revealed the presence of c.1A>C (p.Met1Leu) and c.1187-10G>A mutations in the father and mother, respectively [Table/Fig-4]. The younger sibling 6-years-old showed the presence of both pathogenic mutations similar to the proband.

The presence of compound heterozygous mutations in the LDLR gene and a prior history of hyperlipidemia confirmed the genetic diagnosis of HoFH in the proband. All three family members also had a personal clinical history of hypercholesterolemia. Based on cholesterol levels and the presence of heterozygous mutations, the parents and younger sibling were categorised as HeFH and HoFH, respectively.

Follow-up: Secondary to genetic studies confirming HoFH, the severity of familial hyperlipidemia, and the persistence of cardiovascular complications, a multidisciplinary team determined that a liver transplant would be the definitive treatment strategy. The liver transplant procedure was conducted nearly eight months post CABG surgery for improved lipid metabolism and a reduction in the risk of recurrent cardiovascular events. The younger sibling, who was also a case of HoFH, was started on statins, and a prophylactic liver transplant was done for him as well. All this was revealed during a follow-up counseling session with the parent. Currently, the proband is normal, and her post-liver transplant course will be further closely monitored to evaluate the impact of the liver transplant on lipid profiles and cardiovascular health.

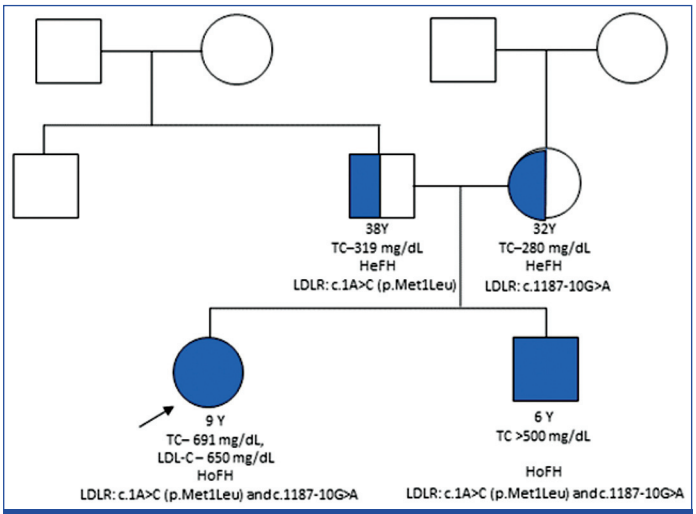
DISCUSSION

Familial Hypercholesterolaemia, MIM# 143890, is an autosomal dominant genetic disorder characterised by high blood cholesterol levels, specifically very high levels of LDL-C. FH cases are at risk of early onset atherosclerosis, Coronary Heart Disease (CHD), stroke, and death. Early identification and treatment of these patients improve their prognosis [5]. The prevalence of HeFH, caused by inheritance of one defective allele, is much higher, approximately 1:250, and HoFH, where the patient has abnormal alleles inherited from both parents, is rare with an estimated frequency of 1:160,000-1:300,000 [6]. Mutations in the Low-density Lipoprotein Receptor gene (LDLR) are most often responsible for the phenotype associated with FH, seen in about 80-85% of cases. Less often, variations in the Apolipoprotein B Gene (APOB) and proprotein convertase subtilisin/kexin type 9 gene (PCSK9) have been documented [7]. Disease severity and age of onset of cardiovascular disease are based on the presence of a homozygous or a heterozygous defect in the gene.

Early identification of these cases using genetic testing and subsequent therapeutic intervention is crucial for disease management and to delay the progression of coronary atherosclerosis. If genetic variants are identified in the patient, then cascade screening in all first-degree family members is the next important step [8].

More than 2600 different variants in LDLR (chr 19p13.1-13.3) have been described in the ClinVar database [5]. The mutation spectrum in the LDLR gene mostly comprises single nucleotide missense and nonsense variations and short indels, and rarely large gene rearrangements or structural variants [7]. The proband

presented with elevated levels of total cholesterol and LDL-C and with premature CHD. The study investigated molecular alterations by WES, and compound heterozygous or biallelic pathogenic variations, c.1A>C (p.Met1Leu) and c.1187-10G>A, were identified in the LDLR gene [Table/Fig-4]. The variation in the start codon leads to the activation of a potential downstream translation initiation site with a new reading frame or non Adenine Uracil Guanine (AUG) initiation [9]. A study conducted by Graca R et al., identified two mutations at the start codon {c.1A>T (p.Met1Leu) and c.1A>C (p.Met1Leu)} in the LDLR gene and concluded based on functional studies that though both variations encode the same amino acid, c.1A>T acts like a null variant with extremely low levels of protein expression in comparison to the c.1A>C variant [10]. The c.1A>C variant has been classified as 'Pathogenic' in the ClinVar database, and the classification has been reviewed by the expert panel.



[Table/Fig-4]: Genogram of homozygous Familial Hypercholesterolemia (FH) patient.

The splice site mutation in intron 8 (c.1187-10G>A) found in the present study is a known pathogenic mutation and is supported by in silico splicing prediction tools. A study from Portugal highlights the importance of functional studies of splice-site mutations in the LDLR gene for the genetic diagnosis of FH [11]. The variation c.1187-10G>A has previously been described in a Chinese case of FH with xanthomas at the age of two months, and eight other family members across three generations with high total cholesterol also had the same mutation [3]. This variation has also been reported in FH in several studies across the globe [12-15].

A cumulative analysis of six Indian studies highlights the fact that the genetic spectrum in approximately 37% of FH cases from India is unknown [16]. This study by Reddy LL et al., also highlighted the importance of large-scale genetic screening for FH in various ethnic groups followed by cascade screening to establish the Indian-specific mutation spectrum. The finding of genetic variants in the LDLR gene, the genotype-phenotype correlation, cascade testing, and subsequent clinical management emphasise the importance of genetic diagnosis.

CONCLUSION(S)

The present case report showcases the power of combining state-of-the-art genomic techniques with advanced variant effect prediction methodologies to unravel the genetic basis of FH. By identifying pathogenic variants in LDLR and their correlation with FH phenotypes, the significance of genetic diagnosis in guiding clinical decision-making can be unveiled. These insights contribute to the understanding of FH pathogenesis and pave the way for tailored therapeutic interventions to mitigate cardiovascular risk in affected individuals.

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