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Cascade screening of a Pakistani consanguineous familial hypercholesterolemia cohort: Identification of seven new homozygous patients

Quratul Ain [a,b], Jaka Sikonja [c,d], Fouzia Sadiq [b,*], Saeed Shafi [e], Jan Kafol [d], Tevz Gorjanc [d], Ursa Sustar [f,g], Jernej Kovac [g], Mohammad Iqbal Khan [h], Muhammad Ajmal [a], Urh Groselj [c,d,**]

[a] Translational Genomics Laboratory, Department of Biosciences, Faculty of Health Sciences, COMSATS University Islamabad, Islamabad, Pakistan
[b] Shifa Tameer-e-Millat University, Islamabad, Pakistan
[c] Department of Endocrinology, Diabetes and Metabolic Diseases, Division of Internal Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia
[d] Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[e] Department of Anatomy, Shifa Tameer-e-Millat University, Islamabad, Pakistan
[f] Department of Endocrinology, Diabetes, and Metabolic Diseases, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia
[g] Clinical Institute of Special Laboratory Diagnostics, University Children's Hospital, University Medical Centre Ljubljana, Ljubljana, Slovenia
[h] Department of Vascular Surgery, Shifa International Hospital, Shifa Tameer-e-Millat University, Islamabad, Pakistan

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ABSTRACT

Background and aims: Familial hypercholesterolemia (FH) is a genetic disorder characterized by elevated low-density lipoprotein cholesterol (LDL-C) levels from birth, significantly increasing the risk of premature cardiac events and mortality. In Pakistan, despite the potential burden of FH, comprehensive studies evaluating its genetic characteristics, cascade screening significance, and lipoprotein (a) [Lp(a)] levels remain scarce. Understanding these factors is crucial for effective diagnosis, risk assessment, and management of FH in the Pakistani population.

Methods: After the identification of index case with clinical homozygous FH, characterized by high LDL-C and high Lp(a) levels together with a positive personal and family history of cardiovascular disease, a cascade screening of 66 relatives from a consanguineous family was performed. Blood samples were obtained from all subjects for biochemical and genetic analysis. Simon Broome criteria was applied on children for clinical FH diagnosis. Dutch Lipid Clinic Network scores were calculated for individuals aged ≥16years. Genetic screening was performed using next-generation sequencing to analyse all coding regions and exon-intron borders of the following genes: *ALMS1, APOA1, APOB, APOA5, APOC2, APOC3, APOE, ABCA1, ABCG5, ABCG8, CREB3L3, GPIHBP1, LDLR, LDLRAP1, LIPA, LMF1, LPL*, and *PCSK9*. The identified variants were confirmed using Sanger sequencing.

Results: Cascade screening identified seven homozygous and 25 heterozygous FH patients with pathogenic variant in the *LDLR* gene (NM_000527.5: c.2416dupG: p. Val806GlyfsTer11). Additionally, heterozygous variants of uncertain significance were identified in 4 other subjects.

Conclusion: This study underscores the high effectiveness of cascade screening in consanguineous families and societies that could lead to early detection and prevention.

1. Introduction

Familial hypercholesterolemia (FH, OMIM# 143890) is an autosomal co-dominant inherited disease characterized by high cholesterol levels, leading to cutaneous cholesterol deposition in the form of xanthomas and significantly contributes to premature cardiovascular events [1,2]. Three major genes are commonly associated with the pathogenesis of FH: pathogenic variants in the low-density lipoprotein receptor

* Corresponding author: Shifa Tameer-e-Millat University, Pitras Bukhari Road, H-8/4, Islamabad, 4400, Pakistan.
** Corresponding author: Department of Endocrinology, Diabetes, and Metabolic Diseases, University Children's Hospital, University Medical Centre Ljubljana, Bohoriceva ulica 20, Ljubljana, Slovenia.
E-mail addresses: fsadiq09@gmail.com, director.research@stmu.edu.pk (F. Sadiq), urh.groselj@kclj.si (U. Groselj).

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(LDLR) gene account for more than 85 % of cases, apolipoprotein B (*APOB*) for about 10 % of cases, and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) for less than 5 % of cases [3,4]. Loss-of-function variants in the *LDLR* and *APOB* whereas gain-of-function variants in the *PCSK9* result in higher serum concentrations of LDL-C and lead to FH [5,6]. These genetic variants lead to dysfunction in the LDLR, which results in reduced uptake of low-density lipoprotein cholesterol (LDL-C) and causes higher levels of LDL-C in the blood [7].

Heterozygous FH (HeFH) occurs when individuals inherit one mutated allele for FH from one parent and one normal allele from the other parent, leading to significantly elevated LDL-C and an increased risk of premature cardiovascular disease [8]. Homozygous FH (HoFH) is more severe and occurs when an individual inherits two mutated alleles, one from each parent. In HoFH, extremely high LDL-C levels are present from birth and represent high atherosclerotic cardiovascular disease (ASCVD) risk [9,10]. Clinical signs typically seen in adults with FH are rare in younger individuals, yet it is crucial to detect this condition early in children and adolescents [11]. Certain criteria such as Dutch Lipid Clinic Network (DLCN) score, Simon-Broome diagnostic criteria, and MEDPED are commonly used for the clinical diagnosis of FH, where the diagnosis is primarily based on clinical features, such as high levels of LDL-C, family history of cardiovascular disease and visual signs like xanthomas and corneal arcus [12–14].

The global prevalence of HeFH is 1:313, while HoFH is prevalent in 1:400,000 [15,16]. Globally >90 % of FH cases are not diagnosed and treated due to a lack of awareness [17–19]. Unfortunately, FH is also often not diagnosed in Pakistan [20] and is either not treated or undertreated, and very few variants have been reported from the country [21–24]. To diagnose FH, genetic testing is crucial, as it aids in confirming the diagnosis and assessing the risk of cardiovascular complications. Cascade screening is a diagnostic process used to examine the presence of pathological variants in first or higher-degree relatives of the index cases [25]. In this study, we present the application of cascade genetic screening principles on 67 subjects, an index case and her 66 relatives from a larger consanguineous Pakistani family to identify individuals at risk for FH.

2. Materials and methods

2.1. Study design

The index case was a 5-year-old female from Punjab, Pakistan, presenting with tendinous xanthomas, LDL-C level of 20.48 mmol/L and Lp (a) of 182 mg/dL and a prior medical history of cardiovascular disease. Her DLCN score without genetics was 17, indicating definite FH. Due to a history of cardiovascular complications and intermarriages among cousins over seven generations, a decision was made to screen her close and distant family members. The study was approved by the institutional review board and ethics Committee of Shifa Tameer-e-Millat University Islamabad, Pakistan (033-523-2019). This study was conducted in accordance with the ethical guidelines established by the Declaration of Helsinki. This screening included 67 subjects comprising an index case and her 66 available relatives, all of whom provided informed consent for cascade genetic screening.

2.2. Lipid profile and Lp(a) testing

Venous blood was collected from all study subjects for genetic testing, lipid profile, and Lp(a) testing. LDL-C, triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were measured by direct homogenous spectrophotometric assay (Alinity c, Abbott, USA). Lp(a) levels were assessed using a turbidimetric immunoassay (Alinity c, Abbott, USA).

2.3. Calculation of DLCN score

Personal and family histories were recorded through interviews and clinical examinations were performed, which were then used to calculate the DLCN score for individual aged >16years [26,27]. DLCN criteria were used without incorporating genetic criteria in the calculation.

2.4. Simon Broome criteria

For individuals aged under 16 years, the Simon Broome criteria were utilized to assess the clinical diagnosis of FH in children. This evaluation was conducted based on clinical and biochemical parameters, without incorporating genetic data [28].

2.5. DNA extraction and genetic screening

DNA extraction was performed for the 67 subjects, at the Shifa Tameer-e-Millat University Islamabad Pakistan, using a DNA extraction kit (Flexigene, Qiagen, Hilden Germany, Catalogue No./ID: 51204). DNA samples were shipped at room temperature to our research collaborators in Slovenia where genetic analysis was performed using next-generation sequencing (NGS). All the coding regions of genes associated with dyslipidaemia including intron exon borders were sequenced using xGen® Lockdown® NGS Probes. NGS was performed on a MiSeq sequencer (Illumina, USA) using the MiSeq Reagent Kit. The following genes were included in the analysis: *ABCA1, ABCG5, ABCG8, ALMS1, APOA1, APOA5, APOB, APOC2, APOC3, APOE, CREB3L3, GPIHBP1, LDLR, LDLRAP1, LIPA, LMF1, LPL* and *PCSK9*. Sanger sequencing was used for the validation of pathogenic variants identified through NGS [29].

2.6. Statistical analysis

Data were gathered and analysed utilizing Microsoft Office Excel 365 (Microsoft Corporation, USA) and SPSS Statistics, version 26.0 (IBM, USA). Mean, SD, and medians were calculated for lipid profile parameters and Lp(a). Subgroup analysis was performed according to age and gender by independent T-test and one-way ANOVA. Assumptions underlying independent *t*-test/one-way ANOVA including normality and homogeneity of variance were assessed. While the Mann-Whitney *U* test and Kruskal Wallis test were employed for Lp(a) subgroup analysis. Two-sided statistical tests were used for all comparisons and a p *value* < 0.05 was considered statistically significant.

3. Results

3.1. General characteristics of the cohort

The study included two first-degree relatives (3 %), 10 s-degree relatives (15 %), and 54 third-degree or higher-degree relatives (82 %) of the index case. In the study, there were a total of 67 subjects including index case, comprising 38 males (57 %) and 29 females (43 %). The mean age ± standard deviation (SD) of the subjects in the cohort was 29.9 ± 17.9 years.

Twenty-Four (35.8 %) subjects were children under 18 years of age. Six subjects (9 %), including the index case, had a personal history of ASCVD, with manifestations including acute coronary syndrome (n = 4, all with documented myocardial infarction), history of surgical revascularization via CABG (n = 3), and cerebrovascular events (n = 2). Nine subjects (13 %) had xanthomata, and one subject (1.5 %) had corneal arcus. Among the 67 individuals, 04 (6 %) had diabetes mellitus and 1 (1.5 %) had thyroid disease. Additionally, 18 subjects (27 %) were using lipid-lowering therapy (LLT) at the time of screening including 10 children (56 %) and 8 adults (44 %). Of the 18 subjects receiving LLT, 12 (67 %) patients were using rosuvastatin [median daily dose 20 mg, range (minimum-maximum) 10–20 mg] combined with ezetimibe (daily

dose 10 mg), whereas the remaining 6 (33 %) were taking atorvastatin [median daily dose 40 mg, range (minimum-maximum) 10–80 mg] along with ezetimibe (daily dose 10 mg). Thirteen patients (13/18) were receiving a high-intensity statin regimen (rosuvastatin 20–40 mg or atorvastatin 40–80 mg). One patient was also receiving a PCSK9 inhibitor (evolocumab 140 mg every two weeks). Despite intensive LLT, none of the 18 patients on LLT achieved the guideline-recommended targets of LDL-C <1.8 mmol/L and <1.4 mmol/L. Additionally, one patient (1.5 %), who was the first cousin of the index case's father had undergone LDL apheresis.

3.2. Lipid profile and Lp(a) results

Table 1 shows the mean ± SD values of TC, LDL-C, TG, HDL-C and Lp (a). The mean TC value measured for all subjects was 7.1 ± 4.4 mmol/L, while HDL-C and LDL-C were 0.9 ± 0.2 mmol/L and 5.3 ± 4.1 mmol/L, respectively. TC and LDL-C levels were higher in females as compared to males (8.7 ± 5.8 vs. 4.2 ± 2.2; $p = 0.01$, 6.7 ± 5.3 vs. 4.2 ± 2.2; $p = 0.01$ respectively). TG levels were higher in adults compared to children (2.7 ± 1.7 vs. 1.4 ± 0.8 mmol/L; $p < 0.0001$).

The median Lp(a) level was 19.8 (IQR = (8.5–43.7) mg/dL. Compared to the males, the median Lp(a) levels were higher in females [14.8 (7.4–37.3) vs 34.5 (13.2–68.8) mg/dL; $p = 0.06$]. Fourteen of the subjects (21 %), including the index case, had Lp(a) levels greater than 50 mg/dL, while 13 subjects (19 %) had Lp(a) levels between 30 and 50 mg/dL.

3.3. Clinical FH diagnosis through DLCN scores

According to DLCN scoring applied on 45 subjects aged ≥16years, 5 (11.1 %) subjects had definite FH, 5 (11.1 %) had probable FH, 15 (33.3 %) had possible FH, and 20 (44.4 %) were less likely to have FH (non-FH). Subjects with definite FH had the highest mean values of LDL-C at 9.8 ± 3.8 mmol/L, and those with probable FH had the second-highest values at 5.7 ± 1.6 mmol/L. Lp(a) levels were also the highest in definite FH subjects at 65.7 (20.1-94.2) mg/dL. The one-way ANOVA analysis confirmed that TC and LDL-C significantly differ among FH classes (Table 2).

Table 1
Variations in lipid profile parameters and Lp(a) levels across gender and age groups with descriptive analysis.

Variables		Mean ± SD	*p-value*
TC (mmol/L)	Overall	7.1 ± 4.4	
	Gender		
	Male	4.2 ± 2.2	*0.01*
	Female	8.7 ± 5.8	
	Age groups		
	Children	7.9 ± 6.4	*0.25*
	Adults	6.6 ± 2.7	
LDL-C (mmol/L)	Overall	5.3 ± 4.1	
	Gender		
	Male	4.2 ± 2.2	*0.01*
	Female	6.7 ± 5.3	
	Age groups		
	Children	6.3 ± 5.7	*0.13*
	Adults	4.7 ± 2.6	
TG (mmol/L)	Overall	2.3 ± 1.6	
	Gender		
	Male	2.4 ± 1.8	*0.30*
	Female	2.1 ± 1.3	
	Age groups		
	Children	1.4 ± 0.8	*<0.0001*
	Adults	2.7 ± 1.7	
HDL-C (mmol/L)	Overall	0.9 ± 0.2	
	Gender		
	Male	0.8 ± 0.1	*0.93*
	Female	0.9 ± 0.3	
	Age groups		
	Children	0.9 ± 0.3	*0.90*
	Adults	0.9 ± 0.2	
Lp(a) (mg/dL) Median (IQR)	Overall	19.8 (8.5–43.7)	
	Gender		
	Male	14.8 (7.4–37.3)	*0.06*
	Female	34.5 (13.2–68.8)	
	Age groups		
	Children	19.6 (7.7–43.1)	*0.88*
	Adults	19.8 (9.7–46.4)	

3.4. Diagnosis of FH in children through Simon Broome criteria

Among the 22 patients aged <16 years, 5 (22.7 %) were classified as having definite FH, 6 (27.3 %) as possible FH, and 11 (50.0 %) as unlikely FH. The mean TC levels were highest in the definite FH group at 19.4 ± 4.4 mmol/L, compared to possible and unlikely FH group ($p < 0.0001$). Similarly, the mean LDL-C was 16.7 ± 3.6 mmol/L in the definite FH group, while the possible FH and unlikely FH groups had mean LDL-C levels of 5.4 ± 0.9 mmol/L and 2.6 ± 0.4 mmol/L, $p < 0.0001$). In contrast, TG levels showed no statistically significant difference across groups, with a mean of 1.7 ± 0.3 mmol/L for definite FH, 0.9 ± 0.3 mmol/L for possible FH, and 1.6 ± 1 mmol/L for unlikely FH ($p = 0.14$). Median Lp(a) levels were significantly higher in the definite FH group at 42.3 (32.8–231.7) mg/dL, compared to 44.3 (14.4–72.3) mg/dL in the possible FH group and 7.4 (3.1–22.2) mg/dL in the unlikely FH group ($p = 0.01$; Table 2).

3.5. Genetic screening results

NGS was successful for 64 samples out of 67. Thirty-two (32/64) subjects were positive for a pathogenic variant in *LDLR* NM_000527.5: c.2416dupG (p. Val806GlyfsTer11). Out of these 32 subjects, 7 were HoFH cases. Five of the HoFH were children (4 females including the index case and 1 male) and 2 were adult females. The remaining 25/32 *LDLR*-positive variants were heterozygous, including 7 children (5 males and 2 female) and 18 adults (9 females including the mother of the index case and 9 males including the father of the index case) as shown in Fig. 1.

Three (03/64) subjects of the cohort (Mean ± SD DLCN score = 3.3 ± 2.5) were found negative for any pathogenic variant in the genes included in the panel. Heterozygous variants with uncertain significance (VUS) were identified in four (4/64) subjects in ATP-binding cassette sub-family G member 5 gene (*ABCG5)*, *APOB* and cAMP responsive element binding protein 3 like 3 (*CREB3L3)* genes (Fig. 2).

Out of 7 homozygous HoFH cases, 6 had manifestation of xanthomas (85.7 %). TC, LDL-C, HDL-C and Lp(a) between HoFH, HeFH, VUS and subjects with no pathogenic were found to be significantly different (Table 3).

3.6. Clinical diagnosis of FH for NGS failed samples

Out of 67 patients screened via NGS, 3 failed the sequencing. Among these, 1 was a female child, who was unlikely to have FH based on the Simon Broome criteria. The other 2 failed NGS were adult males. According to the DLCN criteria, one patient was diagnosed with definite FH, receiving a score of 11, while the other male was classified as having possible FH with a DLCN score of 4.

4. Discussion

FH patients are born with higher levels of LDL-C which increases their risk of premature cardiac events and deaths. This risk is inherited from their parents in an autosomal co-dominant pattern [1,7]. This study discusses genetic characteristics, the importance of cascade screening, and Lp(a) levels in a Pakistani FH cohort. Implementing a genetic cascade screening program for first-degree relatives starting from an index case could effectively identify individuals at high cardiovascular risk, especially in consanguineous families [30,31].

Table 2
Levels of TC, LDL-C, TG, HDL-C and Lp(a) across all FH classes based on Simon Broome criteria and DLCN score.

Variables	Definite FH	Probable FH	Possible FH	Unlikely FH	*p-value*
DLCN Score (n = 45; Age ≥ 16years)	n = 5	n = 5	n = 15	n = 20	
TC (mmol/L) Mean ± SD	12 ± 3.4	7.5 ± 2	6.8 ± 1.6	4.8 ± 0.9	*<0.0001*
LDL-C (mmol/L) Mean ± SD	9.8 ± 3.8	5.7 ± 1.6	4.9 ± 1.1	3 ± 0.8	*<0.0001*
TG (mmol/L) Mean ± SD	1.9 ± 1.1	2.4 ± 1.2	2.4 ± 1.3	3.2 ± 2.2	*0.36*
HDL-C (mmol/L) Mean ± SD	0.9 ± 0.2	1.1 ± 0.2	0.9 ± 0.2	0.9 ± 0.3	*0.65*
Lp(a) (mg/dL) Median (IQR)	65.7 (20.1–94.2)	14.6 (10.8–80.9)	20.8 (10.8–63.5)	12.2 (6.8–36.3)	*0.07*[a]
Simon Broome Criteria (n = 22; Age < 16years)	n = 5		n = 6	n = 11	
TC (mmol/L) Mean ± SD	19.4 ± 4.4	NA	6.4 ± 1.1	4.1 ± 0.5	*<0.0001*
LDL-C (mmol/L) Mean ± SD	16.7 ± 3.6	NA	5.4 ± 0.9	2.6 ± 0.4	*<0.0001*
TG (mmol/L) Mean ± SD	1.7 ± 0.3	NA	0.9 ± 0.3	1.6 ± 1	*0.14*
HDL-C (mmol/L) Mean ± SD	0.6 ± 0.1	NA	1 ± 0.1	1.1 ± 0.2	*<0.0001*
Lp(a) (mg/dL) Median (IQR)	42.3 (32.8–231.7)	NA	44.3 (14.4–72.3)	7.4 (3.1–22.2)	*0.01*[a]

[a] *p value* is calculated by using Kruskal-Walli's test.

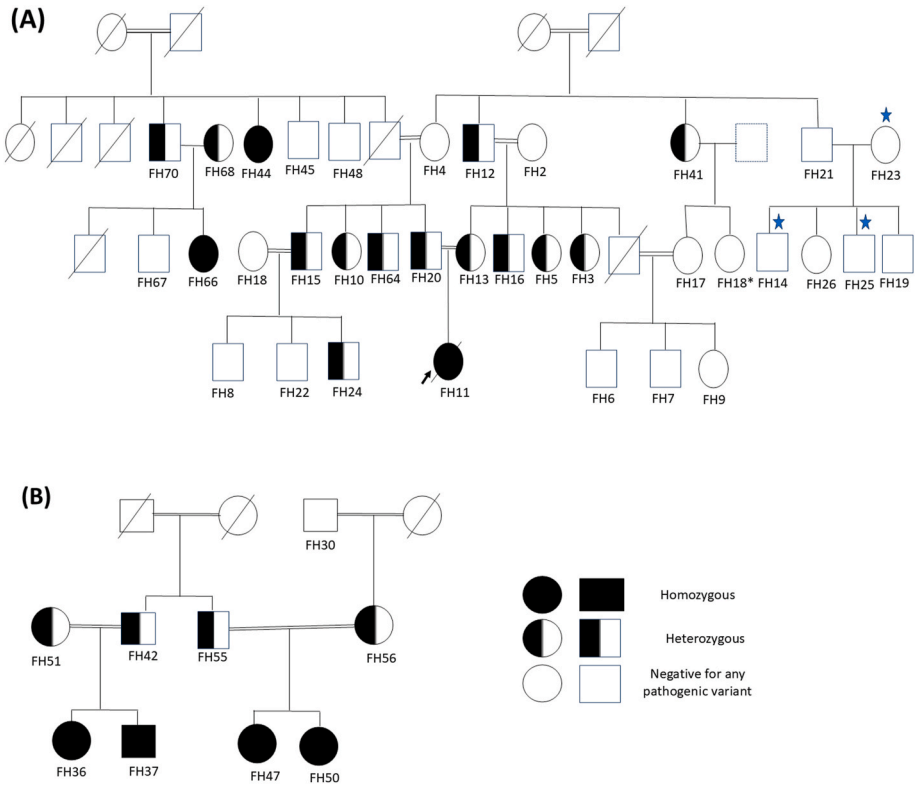

Fig. 1. Pedigree chart of a consanguineous Pakistani family with familial hypercholesterolemia. Panel A depicts first-degree and second-degree relatives of the index case (homozygous FH), while Panel B represents third-degree or higher relatives. Individuals with dotted outlines in pedigree boxes were not available for genetic screening. FH 18* appear two times in the pedigree. Stars (★) indicate carriers of heterozygous variants of uncertain significance (VUS) in the APOB and ABCG5 genes.

Cascade testing targets specific populations with established genetic predispositions to FH, such as families with a history of the condition, while opportunistic testing, with its broader reach beyond familial connections, has the potential to capture individuals who may not have an obvious family history of FH [32], however, the combination of both could yield a higher identification of individuals with FH. We identified 7 cases of HoFH and 25 of HeFH through genetic testing. In this study, we report seven HoFH cases from a single family in Pakistan, representing approximately 1 % of the 751 HoFH patients included in the HoFH International Clinical Collaboration (HICC) registry [33]. This underscores how the cascade screening program can identify diverse cases within consanguineous families. Similar findings have been reported in other regions globally, highlighting the universal importance of cascade screening in identifying FH variants and informing effective management approaches [34–40]. Pathogenic variant detected in the *LDLR* gene has been previously documented in both Pakistani and global populations [21,41–44], with established links to severe FH phenotypes. While other VUS variants have also been previously reported in the genetic databases.

In Pakistan, Lp(a) levels are not routinely measured. To the best of our knowledge, this is the first study in Pakistan to report on the Lp(a) levels in FH subjects. Lp(a) testing is crucial in FH subjects to help assess the risk of earlier cardiac events [13]. If the subjects regardless of their FH diagnosis, have >50 mg/dL Lp(a), then they have a 2-3-fold higher risk of getting a heart attack [45]. Fourteen subjects (21 %) in our study had Lp(a) levels greater than 50 mg/dL, including the index case who was HoFH with an Lp(a) level of 182 mg/dL.

According to World Population Review 2024, Pakistan has the highest rate of consanguineous marriages (61.2 %), primarily due to deeply rooted cultural traditions, social preferences, and the desire to

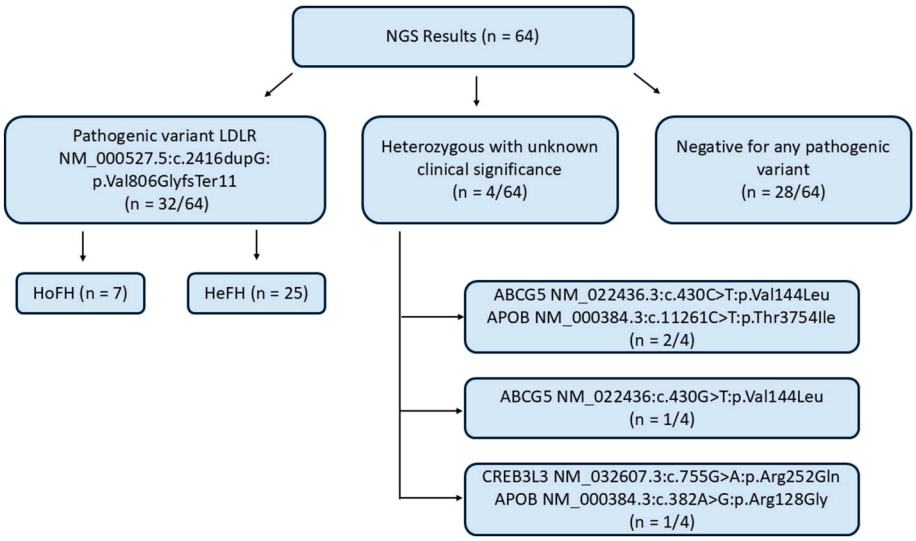

Fig. 2. Next-generation sequencing (NGS) results (n = 64) *LDLR*; low-density lipoprotein receptor, VUS; variant of unknown significance, HoFH; homozygous familial hypercholesterolemia, HeFH; heterozygous familial hypercholesterolemia, *ABCG5*; ATP-binding cassette sub-family G member 5 gene, *APOB;* apolipoprotein B, *CREB3L3;* cAMP responsive element binding protein 3 like 3.

Table 3
Clinical characteristics of the subjects based on genetic characterisation. (n = 64).

Variables	Genetic Characteristic				
	HoFH (n = 7)	HeFH (n = 25)	VUS (n = 4)	Negative for pathogenic variant (n = 28)	*p value*
Children (<18 years), n (%)	5 (71.4)	7 (28.0)	3 (75)	8 (28.6)	
Adults (≥18 years), n (%)	2 (28.6)	18 (72.0)	1 (25)	20 (71.4)	
Xanthoma, n (%)	6 (85.7 %)	1 (4.0)	0 (0)	1 (3.6)	
Arcus, n (%)	1 (14.3)	0 (0)	0 (0)	0 (0)	
ASCVD history, n (%)	3 (42.9 %)	2 (8)	0 (0)	1 (3.6)	
Mean Age (Years)	16.1 ± 12.9	33.1 ± 16.1	17.1 ± 15.1	32.3 ± 19.1	*0.05*
TC (mmol/L)	18 ± 4.8	7.4 ± 1.8	4.2 ± 0.7	4.7 ± 1.1	*<0.0001*
LDL-C (mmol/L)	15.6 ± 4.1	5.5 ± 1.5	2.8 ± 0.6	3 ± 1	*<0.0001*
TG (mmol/L)	1.4 ± 0.6	2.3 ± 1.4	1.1 ± 0.4	2.7 ± 2	*0.15*
HDL-C (mmol/L)	0.6 ± 0.1	0.9 ± 0.2	1.2 ± 0.1	1 ± 0.3	*0.01*
Lp(a) (mg/dL) Median (IQR)	91.0 (34.5–182.0)	20.8 (12.9–65.6)	27.9 (6.0–42.7)	11.5 (6.6–31.9)	*0.01*[a]

[a] *p value* is calculated by using Kruskal-Walli's test.

maintain family ties and property within families [46]. One in 409 people in the Pakistani population has FH [47] which emphasizes the importance of implementing cascade screening for the early identification and management of FH patients in Pakistan. This approach can help prevent cardiac events and early death and reduce the burden of CVD on the Pakistani population.

4.1. Current state of lipid management in Pakistan

Lipid management in Pakistan is centred in tertiary care hospitals and cardiology units in urban areas, while rural areas rely on general practitioners with limited resources. Statins, especially atorvastatin and rosuvastatin with or without ezetimibe and fenofibrate are primary treatments. Advanced options, such as PCSK9 inhibitors and LDL apheresis are inaccessible due to high costs and PCSK9 inhibitors must be imported on case-to-case basis. With no universal health insurance, most patients pay out-of-pocket, and free or subsidized medications are limited to availability in government hospitals [19]. These challenges, combined with low awareness and inadequate routine lipid profiling, result in poor management of lipid disorders.

4.2. Limitations

A limitation of this study is the lack of systematic imaging data. While our focus was genetic cascade screening, future studies would benefit from incorporating comprehensive imaging protocols to better correlate genotype findings with coronary status. This would provide valuable insights into genotype-phenotype relationships in this population. Cascade screening may overlook a significant portion of the undetected FH population by focusing solely on relatives of index cases. To address this limitation, combining novel strategies such as systematic and population-wide screening with cascade screening could cost-effectively enhance overall detection rates. Additionally, three samples failed for NGS, potentially due to issues such as DNA quality.

4.3. Recommendations

This study has the potential to significantly enhance FH screening and lipid management in Pakistan by addressing key gaps in awareness, detection, and treatment strategies. By highlighting the prevalence and clinical characteristics of FH, it can raise awareness among healthcare providers and policymakers about this underdiagnosed condition. The study also emphasizes for implementing systematic cascade screening programs and accessible genetic testing to improve early detection in families with at risk of FH and CVDs. The findings also underscore the need for localized lipid management guidelines tailored to healthcare system of Pakistan. By addressing these challenges, the study aims to reduce the burden of cardiovascular diseases in Pakistan and improving overall health outcomes.

5. Conclusion

Cascade screening proves essential in identifying FH cases. Implementing comprehensive screening strategies could possibly mitigate the burden of cardiovascular disease and prevent premature deaths in the Pakistani population, particularly in specific consanguineous societies.

CRediT authorship contribution statement

Quratul Ain: Writing – original draft, Methodology, Writing – review & editing, Investigation. **Jaka Sikonja:** Writing – original draft, Methodology, Writing – review & editing. **Fouzia Sadiq:** Conceptualization, Data curation, Methodology, Supervision, Writing – review & editing. **Saeed Shafi:** Data curation, Writing – review & editing. **Jan Kafol:** Data curation, Writing – original draft, Writing – review & editing. **Tevz Gorjanc:** Data curation, Writing – review & editing. **Ursa Sustar:** Methodology, Investigation, Writing – review & editing. **Jernej Kovac:** Methodology, Investigation, Writing – review & editing. **Mohammad Iqbal Khan:** Supervision, Writing – review & editing. **Muhammad Ajmal:** Supervision, Writing – review & editing. **Urh Groselj:** Conceptualization, Writing – review & editing.

Data sharing statement

The dataset from the current study is not publicly available.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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