Genetic Profile of Premature Coronary Artery Disease Associated With Dyslipidemia: A Preliminary Study in Tertiary Hospitals

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Original Article

ABSTRACT

Background

Familial Hypercholesteolemia (FH) represents significant risk for premature Coronary Artery Disease (CAD) development. Testing for causative mutations is the gold standard diagnostic test for FH. This study aimed to estimate the prevalence of genetically-confirmed Familial Hypercholesterolemia in premature Coronary Artery Disease patients in an Egyptian cohort.

Methods:

An Observational Analytical Cross-sectional study was conducted at Kobri Alqoba military hospital in Egypt in 2022, including 47 participants. All participants underwent Next generation sequencing for 7 genes linked with FH development in 47 patients with Premature CAD.

Results:

9 Variants of Uncertain Significance (VUS) were found in LDLR, APOB, and APOE genes in 20 of 47 (42.5%) patients. Four variants; LDLR (rs141673997), APOB (rs772173177), APOB (rs41288783), and APOB (NM_000384.3) were novel. No pathogenic or likely pathogenic variants were detected. The prevalence of definite/probable FH diagnosed by DLCN criteria were 14.8% (7/47). DLCN criteria detected only 1 patient with a VUS (14.3%).

Conclusions:

DLCN scoring system is not an accurate screening tool for Familial Hypercholesterolemia and genetic testing for accurate diagnosis and management of FH is essential for high risk cases.

Key Words: DLCN, Familial Hypercholesterolemia, genetic testing, premature Coronary Artery Disease.

Received: 12 December 2024, **Accepted:** 13 january 2025

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ISSN: 2812-5509, 2024

INTRODUCTION

Coronary Artery Disease (CAD) occurs through plaque deposition over time, leading to a progressive obstruction of coronary arteries.[1] It is the number one cause of death globally.[2] Premature Coronary artery disease (PCAD) is CAD occurring before 55 years in males and 65 years in females.[3]

Familial hypercholesterolemia (FH) is high low-density lipoprotein in plasma that runs in families. FH was found to be associated with an earlier onset of CAD.[4] FH can be polygenic, autosomal dominant, or autosomal recessive.

The prevalence of FH has been estimated to be around 1 in 300 individuals, although varying prevalence rates

DOI: 10.21608/ARCMED.2025.343790.1071

have been found in different populations.[5] The prevalence of FH in premature CAD is around 1 in 7 patients.[6] No genetic studies on FH were published for Egyptian patients until now.

Early detection of FH would help lower incidence rates of premature CAD through cascade screening and early lipid control of patients with causative mutations.[7]

This study was conducted with a hypothesis that premature CAD patients would show a high prevalence of mutations in FH genes.

In this study, gene panel sequencing was performed for 7 genes related to FH; LDLR, PCSK9, APOE and APOB, which are related to the development of autosomal dominant familial hypercholesterolemia, and ABCG5, ABCG8 and LDLRAP1 which are related to the development of autosomal recessive familial hypercholesterolemia.[8,9]Genetic testing remains the gold standard for diagnosing FH. [5]

MATERIALS AND METHODS

Research design and setting

This is an Observational Analytical Cross-sectional study. Patients were selected for the study from patients at Kobri El Qobba Cardiology hospital's wards, critical care units and emergency unit in Cairo from June 2022 to January 2023. NGS (Next Generation sequencing) was performed at the Egypt Centre for Research and Regenerative Medicine in Cairo.

Participants

Inclusion criteria were males <55 and females <60 years of age with CAD. CAD was diagnosed using coronary angiogram. Patients with obstructive (>50% stenosis on coronary angiogram) and non-obstructive CAD (<50% stenosis) were included.[10] Those excluded from the study were those with thyroid dysfunction, hepatic or renal disease.

Data collection tools

- Patient Interview (History and physical examinations)
- Lipid profile
- Next generation Sequencing

Procedures

Patients with confirmed premature CAD were selected, 48 patients were included, procedures of participation were explained to them, a written informed consent was taken from each patient, a clinical sheet was used for collecting relevant history, two peripheral venous blood samples were collected per patient; one for a full lipid profile and another in Ethylenediaminetetraacetic acid (EDTA) collection tubes for DNA extraction.

Clinical data collected from the patients included age at disease onset, consanguinity, weight and height used for BMI calculation, presence of CAD risk factors; family history of premature CAD or FH, smoking, hypertension and diabetes and lipid lowering drugs intake, types and dosage. DLCN score was calculated for each patient as a scoring system for FH.[11]

Levels of LDL were calculated according to the Friedewald formula.[12] On treatment LDL-C levels for CAD patients of over 55 mg/dl (Target on-treatment LDL levels for CAD patients) were used to determine the presence of hypercholesterolemia.

Genomic DNA was extracted using Chemagic DNA blood 400 kit h96 (Revvity, Baesweiler, Germany) according to the manufacturer's protocol. DNA concentration and quality were assessed using Nanodrop and Qubit Flurometer (Thermo Fisher Scientific, Waltham, Massachussets). Qubit dsDNA BR Assay kit was used in this step (Thermo Fisher Scientific, Waltham, Massachussets).

Microfluidic electrophoretic separation of nucleic acids was conducted on LabChip GXII Touch using the Genomic DNA reagent kit (Revvity, Baesweiler, Germany). Library preparation was performed using the DNA prep with enrichment kit (Illumina, San Diego, California), followed by DNA quantitation with Qubit dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, Massachussets). Library quality was assessed using DNA NGS 3K Reagent kit (Revvity, Baesweiler, Germany) on the LabChip GXII Touch platform. Quality was assessed following DNA fragmentation and after library preparation.

DNA sequencing was performed on the Illumina NextSeq 2000 sequencer using a 2x151 bp protocol on a P1 300 flow cell. TruSight Cardio Sequencing kit (Illumina, San Diego, California), which targets the exons of 174 genes related to different inherited cardiac conditions. These genes include LDLR, APOB, APOE, PCSK9, ABCG5, ABCG8, LDLRAP1.

Alignment and variant calling were performed on NextSeq 2000's onboard DRAGEN secondary analysis system. High quality reads (Q>33 reads/base) were mapped to hg19 (Human Genome 19 reference genome). Samples had a minimum read depth of 52.16x and an average depth of 129.42x. Annotation and variant analysis were done using Varsome v11.15 (https://varsome.com/) and Franklin v.73(https://franklin.genoox.com/clinical-db/home) platforms. The American College of Medical Genetics (ACMG) guidelines were applied in variant scoring and each variant was also assessed based on pathogenicity scores, effect on target protein and conservation scores[13]
Sampling and Sample size

Sampling was done through non-probability convenience sampling. According to one study, the prevalence of genetically confirmed Familial Hypercholesterolemia in patients with CAD is 8.7%.[14] Due to the limited resources, the precision limit could be increased up to 10% but cannot surpass the disease prevalence. Based on these data, the minimum sample size needed at a confidence level of 95% and precision (margin of error) of 8.0% is 48 subjects. This sample size was calculated using Epi INFO version 7.2.4.0 (Centers for Disease Control and Prevention (CDC), Atlanta, Georgia).

Statistical analysis

All statistical analyses were performed using SPSS (Statistical Package for Social Sciences)

version 23 (IBM SPSS Statistics, IBM Corporation, Armonk,

New York).

Normality of data was assessed using Kolmogorov-Smirnov statistical normality test. Quantitative data are presented as mean ± standard deviation (SD) or median (interquartile range [IQR]), unless otherwise specified. Qualitative data are presented as frequency or percentage.

The Chi-square test was used to compare frequencies and differences, while quantitative variables were evaluated using the Student T test or Mann–Whitney U test. Two-sided *p-values* ≤0.05 were considered statistically significant.

Ethical considerations

The study proposal was approved by the Armed Forces College of Medicine Ethical Review Committee (IRB: XX, meeting: January 14th, 2023; serial number: 128). Written informed consent was obtained from all participants before enrollment in the study. The study conformed to the requirements of the Revised Helsinki Declaration of Biomedical Ethics. The policy of data confidentiality was strictly followed.

RESULTS

Characteristics of the study population

This study aimed to study the prevalence of FH in premature CAD cases in a tertiary hospital in Cairo. 48 male patients were studied. Premature CAD in males is defined as the onset of CAD before the age of 55, whereas early CAD is defined as the onset before 40 years. The age of CAD onset in the test sample ranged from 24 to 54 years and the median age of onset was 48 (7) years. Positive first degree family history of premature CAD was detected in 21 patients (43.75%) and 23 (47.91%) had a positive first degree family history of dyslipidemia.

The cohort's risk factors were investigated and it was revealed that 75% of the cohort were smokers, 50% were hypertensive, and 16.6% were diabetic. 15 patients had one risk factor (31.25%), 9 had 2 risk factors (18.75%), 18 had 3 risk factors (37.5%), 2 had 4 risk factors (4.16%) and 4 had no risk factors (8.3%).

Baseline LDL was estimated according to the type and dose of the lipid-lowering treatment before applying the DLCN criteria.(15) LDL levels had a median of 97.50 (49.95) mg/dl, ranging from 29 to 273 mg/dl. Of the 48 individuals, 45 (93.75%) were on lipid lowering agents, according to the treatment protocol, regardless of their lipid profile, since their diagnosis of CAD was confirmed; 44 of which were started on high intensity statins and 1 patient was started on moderate intensity statins with adjuvant fenofibrate and ezetimibe.

Out of the 48 individuals, 6 cases (12.5%) showed target on-treatment LDL levels (<55 mg/dl). 2 other patients (4.16%) had high on-treatment LDL levels (LDL value 160-189) and 5 patients (10.41%) had very high on-treatment LDL levels (LDL value 190 or greater). Table 1 shows the clinical and demographic data of the current study cohort. 2 patients had no data for their lipid profile. These missing data were excluding from analysis.

BMI was calculated for each case; 7 patients (14.6%) were in the healthy weight range (<25), 17 patients (35.4) were in the overweight range (25-29.9), 17 (35.4) were in the class 1 obesity range (30-34.9), 6 (12.5%) were in the class 2 obesity range (35-39.9) and 1 patient (2.1%) was in the class 3 obesity range (>40).

DLCN score was calculated as a predictor for FH, 18 patients (37.5%) had unlikely FH (Score <3), 23 patients (47.92%) had possible FH (Score 3-5), 6 patients (12.5%) had probable FH (Score 6-8) and 1 patient (2.08%) had definite FH (Score > 8).

Table 1: Test sample characteristics (N = 48) Missing (N)

Males n (%)	48 (100%)	-
Age of CAD onset, years – median (IQR)	48 (7)	-
Body mass index - mean ± SD	30.06 ± 4.70	-
Hypertensive - n (%)	23 (47.91)	-
Diabetic - n (%)	18 (37.5)	-
Smoker - n (%)	35 (72.91)	-
Family history of premature CAD - n (%)	21 (43.75)	-
Family history of dyslipidemia - n (%)	23 (47.91)	-
Premature cerebral or peripheral vascular disease - n (%)	6 (12.5)	-
Multi-vessel CAD - n (%)	19 (39.5)	-
Positive consanguinity - n (%)	13 (27)	-
Cholesterol, mg/dl - median (IQR)	169.50 (55)	2
LDL, mg/dl - median (IQR)	97.50 (49.95)	2
HDL, mg/dl - median (IQR)	43.50 (12)	2
Triglycerides, mg/dl - median (IQR)	152 (120.75)	2
On lipid lowering agent – n (%)	45 (93.75)	-
High intensity statins n (%)	44 (91.66)	-

Values are presented as mean ± SD, n (%), or median (IQR).
CAD = Coronary Artery Disease; HDL = High-density lipoprotein; LDL = Low-density lipoprotein.

Genetic analysis

Out of the 48 samples, 1 sample failed quality checks. Out of 47 cases sequenced, 11 variants of uncertain significance (VUS) were found in 20 cases. No pathogenic or likely pathogenic variants were found in any of the cases. The VUS and their evidence are presented in Table 4.

The 9 cases showed variants in 3 genes; LDLR, APOB and APOE and no mutations were found in PCSK9 gene. Case 1 had a missense variant in LDLR with a gnomAD exome frequency of 0.0000716 (PM2) and 15 pathogenic or likely pathogenic reported variants were found in a 49bp region surrounding this variant in exon 10 without reporting of any missense benign variants (PM1 and PP2). The combination of these criteria would lead to the viable suspicion that this variant may fall within the classification of likely pathogenic or pathogenic and warrants further study such as segregation study to aid with a classification.

Case 2 had a single nucleotide insertion variant in a non-coding region, which is not in a splice region (BP7) in LDLR which was not found in gnomAD genome frequencies (PM2). Case 3 had a synonymous single nucleotide variant (BP7) in APOB with no gnomAD genome frequency reported (PM2).

Case 4 had a missense variant in APOB with a gnomAD exome frequency of 0.00047 (PM2) and 7 uncertain, 6 likely benign and 2 benign clinvar reports (BP6).

Case 5 had a synonymous single nucleotide variant (BP7) in APOB with no gnomAD genome frequency reported (PM2). Case 6 had a missense variant which was not found in gnomAD exome frequencies (PM2) but multiple computational prediction tool supported a benign effect on the gene (BP4, aggregated score of 0.053 indicating a benign supporting prediction).

Case 7 represents 6 patients who had a missense variant in APOE with a uniprot pathogenic classification and the following clinvar reports: 1 pathogenic (Before 2015), 1 uncertain significance (Before 2015), 1 risk factor (Before 2015), 2 likely benign (One before and one after 2015) and 2 benign reports (One before and one after 2015) (BP6). This points towards a controversial classification of this variant indicating the need for further study into its classification.

Case 8 had a missense variant in APOE. A different pathogenic variant was found in the same codon (chr19:45412041:G>A) (PM5). Computational prediction tools support a deleterious effect on the gene (Aggregated score 0.714 indicating a pathogenic supporting prediction) (PP3). gnomAD exome allele frequency is 0.01951, which is higher than would be expected for a pathogenic variant (BA1). Variant was observed in a homozygous state in

population databases more than expected for disease (BS2). Clinvar had the following reports: 1 pathogenic (Before 2015), 1 likely pathogenic, 1 VUS, 1 likely benign and 5 benign (All after 2015) (PP5 and BP6). Uniprot classified the variant as pathogenic. The high allele frequency found points towards this VUS being more likely benign than pathogenic.

Case 3 and case 8 represent the same patient in which 2 VUS in 2 different genes related to FH were found.

Case 9 represents 7 patients with the same missense variant in APOE. Clinvar had he following submissions for this variant: 2 pathogenic, 2 likely pathogenic, 2 VUS and 1 risk factor (All after 2015) and 1 submission as a risk factor after 2015 (PP5). It is clear from this evidence that the variants found in cases 1 and 9 show promise as potentially pathogenic/likely pathogenic variants that require further research in order to reach a clear classification.

Cases 10 and 11 represent the same patient with 2 compound heterozygous VUS in ABCG8. Clinvar had the following submissions for case 10's variant: 3 VUS and 1 likely benign and in-silico data support the classification of benign. The variant found in case 11 had 2 Clinvar VUS reports and has conflicting in-silico predictions.

Table 2 Variants of Uncertain Significance in autosomal dominant FH-related genes

Variant number	Gene:codon change (Ref. Seq)	Zygosity	Variant location	GnomAD frequency	Exon/intron	Variant type	ACMG evidence	Number of cases
1	LDLR:c.1546G>A (rs141673997)	Heterozygous	Chr19:11224398:G:A	0.0000716	Exon	Missense .Gly516Ser	PM1, PM2, PP2	1
2	LDLR:c.2390-34dup (rs772173177)	Heterozygous	Chr19:11240150:C:CG	Novel	Exon/intron boundary	Insertion	PM2, BP7	1
3	APOB:c.3279C>T (NM_٠٠٠٣٨٤,٣)	Heterozygous	Chr2:21239364:G:A	Novel	Exon	Synonymous	PM2	1 (same patient as case 8)
4	APOB:c.2981C>T (rs41288783)	Heterozygous	Chr2:21242613:G:A	0.00047	Exon	Missense .Pro994Leu	PM2, BP6	1
5	APOB:c.948A>G (rs41288783)	Heterozygous	Chr2:21256347:T:C	Novel	Exon	Synonymous	PM2, BP7	1
6	APOB:c.2062A>T (NM_000384.3)	Heterozygous	Chr2:21250705:T:A	Novel	Exon	Missense p.Ile٦٨٨Phe	PM2, BP4	1
7	APOE:c.526C>T (NM_000041)	Heterozygous	Chr19:45412079:C:T	0.0615	Exon	Missense p.Arg176Cys	BP6	6
8	APOE:c.487C>T (rs769455)	Heterozygous	Chr19:45412040:C:T	0.01951	Exon	Missense p.Arg163Cys	PM5, PP3, PP5, BA1, BS2, BP6	1
9	APOE:c.388T>C (rs429358)	Heterozygous	Chr19:45411941:T:C	0.138	Exon	Missense p.Cys130Arg	PP5	7
10	ABCG8 (rs150977210)	Compound heterozygous	Chr2:44100940:A:G	0.00031	Exon	Missense	PM2, BP4, BP6	1
11	ABCG8 (rs1394612784)		Chr2:44102415:T:C	0.00000398	Exon	Missense	PM2, PP3	

Chr = Chromosome; rs = reference sequence; LDLR= Low-density Lipoprotein receptor; APOB = Apolipoprotein B; APOE = Apolipoprotein E. Variants were called based on the human genome 19 build.

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Table 3 : Variants of Uncertain Significance in heterozygous state in autosomal recessive FH-related genes

Variant number	Gene (Ref. Seq)	Variant location	GnomAD frequency	Exon / intron	Variant type	Number of affected patients
1	ABCG8 (rs373723529)	Chr2:44079544:G:A	0.0000319	Exon	Missense	1
2	ABCG8 (rs772484840)	Chr2:44101029:G:A	0.00000398	Exon	Missense	1
3	ABCG8 (NM_022437.3)	Chr2:44078915:A:C	Novel	Exon	Missense	1

Chr = Chromosome; rs = reference sequence; LDLR= Low-density Lipoprotein receptor; APOB = Apolipoprotein B; APOE = Apolipoprotein E. Variants were called based on the human genome 19 build.

Table 3: Characteristics of patients with and without FH mutations

	FH mutation (n= 8)	Missing	No FH mutation (n= 39)	Missing	p-value
Age of CAD onset	27.25	-	23.33	-	.460
LDL	22.58	2	23.06	-	.933
HDL	14.08	2	24.37	-	.073
Triglycerides	25.50	2	22.62	-	.616
Cholesterol	22.25	2	23.12	-	.881
Hypertension	3 (37.5)	-	19 (40.4)	-	.336
Diabetes Mellitus	2 (25)	-	15 (31.9)	-	.521

Data is presented as mean rank or n (%). LDL = Low-density Lipoproteins; HDL = High-density Lipoproteins; FH = Familial Hypercholesterolemia

The correlation between genetic and clinical data was made based on the 2 variants with more likelihood of pathogenicity (LDLR, rs373723529 and APOE, rs429358) Those 2 variants are found in 8 (17%) of the patients. Of those 8 patients, 2 showed a multi-vessel disease, 3 were hypertensive, 2 were diabetics, 3 had a positive family history of premature CAD, 6 were smokers, 7 were on high intensity statins and all 8 had optimal or near optimal on-treatment LDL levels but none of them achieved target LDL levels of <55 mg/dl. Characteristics of patients with and without the mutation were compared in Table 3. There were no statistically significant differences in respect to age of CAD onset, lipid profile or existence of relevant comorbidities between those with mutations and those without.

DISCUSSION

This study investigated the prevalence of genetically-confirmed FH in 48 premature CAD patients. LDLR, PCSK9, APOB, APOE, LDLRAP1, ABCG5 and ABCG8 genes were sequenced using NGS.

No pathogenic or likely pathogenic variants were found in any patient and 9 VUS were found in 20 patients (Table 2). Upon thorough analysis considering several factors, 2 variants found in 8 patients showed promise to be rescored as likely pathogenic variants (Chr19:11224398:G:A) and Chr19:45411941:T:C). However further analysis, such as segregation studies, is warranted to prove potential of pathogenicity.

When comparing the clinical characteristics of patients having those VUS and those without (Table 3), it was found that there was no statistically significant difference between the group carrying any of the 2 suspected mutations and the group without the mutations. This supports that the presence of these mutations did not have a significant effect on the age of CAD onset, severity of dyslipidemia or the development of HTN or DM.

The lack of discovery of pathogenic or likely pathogenic variants in our study population could be due to different factors; the cause of premature CAD could be environmental in nature caused by any of the other risk factors in the study cohort diluting the percentage of FH as a primary cause of PCAD. This proposed explanation is due to the fact that 75% of our cases were smokers, 50% were hypertensive and 16.6% were diabetic and most of our patients reported a stressful lifestyle. Each of these factors alone leads to a substantial elevation in CAD risk. It is notable that HTN could be 2ry to Familial Hypercholesterolemia, which may be due to atherosclerosis, release of nitric oxide, or impairment of renal microvasculature.[16]

Another possible explanation is the presence of polygenic etiology of FH. Multiple variants of small effect size in genes associated with LDL metabolism whose combined effect results in the clinical phenotype of FH. Prevalence of PCAD was also found to be very high in the Egyptian population (51% of all CAD).[17] This high burden could be attributed to a higher prevalence of CAD risk factors (Including FH). This may call for the inclusion of a younger age of PCAD than that included in other populations in order to more accurately detect those with FH.

Mutations in other genes related to lipid metabolism and not included in our analysis, such as LIPA and CYP7A1, could also lead to the phenotype of FH. The reason for these genes not being included in this study is a technical one, as these genes were not included in the commercially available inherited cardiac gene panel used for sequencing in the present study.

Issues related to patient selection could berepresented by two main aspects; firstly, recall bias represented by a difficulty in ascertaining an accurate family history of dyslipidemia, presence of tendon xanthomas or arcus cornealis. And secondly, most of our patients were started on high intensity statins upon CAD diagnosis without prior evaluation of lipid profile to measure a baseline LDL level. Both issues lead to inaccurate calculation of a DLCN score which decreases its utility as a patient selection tool for further genetic testing.

Diabetes Mellitus is an independent risk factor for CAD. The presence of patients with DM in our study cohort may have led to the overestimation of the DLCN score. However, in the present study, the absence of DM in most cases with probable or definite DLCN scores points towards DM not acting as a primary cause leading to an overestimated DLCN.

A comparison was made to illustrate the accuracy of detection of FH patients using DLCN. Findings in Table 4 support DLCN being inaccurate in predicting FH mutations and thus, being inaccurate as a patient-selection tool for genetic testing for FH. .

Table 4 demonstrates that 7 of 8 patients carrying VUS fell within the classification of unlikely and probable FH. This analysis was conducted using the Chi-Square test ($X2$ (3, N= 48) = 2.852, p = .415). No statistically significant differences were found regarding DLCN scores between mutation positive and negative cases.

Table 4 : DLCN scores for patients with and without mutations

			Mutation	
	Yes	No		
DLCN	Unlikely	Count	5	12
		% within Mutation	62.5%	30.8%
	Possible	Count	2	21
		% within Mutation	25.0%	53.8%
	Probable	Count	1	5
		% within Mutation	12.5%	12.8%
	Definite	Count	0	1
		% within Mutation	0.0%	2.6%
Total		Count	8	39
% within Mutation			100.0%	100.0%

DLCN = Dutch Lipid Clinic Network

A few studies have investigated the prevalence of FH in different populations using DLCN verses genetic testing. Cui et al discovered that 10 patients out of 225 had genetically confirmed FH (4.4%) and that DLCN successfully detected 4 of them (40%), while Amor-Salamanca et al found 9 out of 103 patients had positive results on genetic testing (8.7%) and DLCN successfully detected 5 of them (55.55%).(14,18) It can be deduced from the previous findings that DLCN on its own cannot accurately diagnose FH and genetic confirmation for patients with suggestive DLCN scores of FH is warranted.

Pre-symptomatic detection of FH is of great benefit to the patient, since this would greatly improve prognosis through early modification of risk factors and lifestyle changes. It will also allow familial risk prediction and cascade screening for the extended families of affected patients. NICE guidelines recommend gene sequencing for patients with DLCN scores above 5 and cascade screening up to the third degree relatives of mutation-positive patients.[19]

Studies have shown that LDL levels vary among patients harboring different types of mutations. One study by Cui et al showed that carriers of LDLR mutations had significantly higher LDL levels than those with APOB mutations (5.72 vs 4.93 mmol/L, respectively) and that 60% of genetically-confirmed FH had LDL levels of over 190 mg/dl.(18) Two other studies by Abul-Husn et al and Khera et al found that only 45% of genetically confirmed FH cases had LDL levels of over 190 mg/dL.(20,21) This points towards LDL being insufficient on its own as a sole diagnostic tool for FH.

Maintaining LDL levels within the desirable range leads to lowered morbidity and mortality NICE guidelines recommend for FH patients with a target reduction of the baseline LDL levels of at least 50%.[19,22]

In Egypt, this is the second study to investigate the prevalence of FH in premature CAD. The first study by Reda et al was based on the electronic data available on a cross-sectional nationwide study called the CardioRisk project. DLCN score was calculated for 2743 patients and it was revealed that 4 patients had definite FH (0.1%), 7 patients had probable FH (0.25%), 461 patients had possible FH (16.8%) and 1271 patients had unlikely FH (82.85%).(23) These findings coincide with the findings of this study that the majority of patients with CAD would fall under the diagnosis of unlikely or possible FH when only relying on the DLCN score.

This is among the very early pilot studies in Egypt, and the first in Cairo, to conduct genetic testing for FH mutations in CAD patients to date. Among the limitations of the current study is that it was conducted in a single center and on a small sample size of 48 male patients.

Limitations of the study

The main limitation of the study involved the precise calculation of DLCN scores for all study participants. Issues that arose included limited patients' knowledge of their family history of tendinous xanthomas, arcus cornealis, and dyslipidemia. Also, most patients were already on high-intensity statins with no knowledge of their baseline LDL levels. This led to using estimated LDL levels to calculate DLCN scores, therefore DLCN scores presented in this study are most likely to be underestimated

Participants of this study were males only. This is attributed to the fact that CAD is more prevalent in females, which was translated into a few number of females meeting the inclusion criteria during the data collection period. Since Familial Hypercholesterolemia is inherited in an autosomal dominant or autosomal recessive patterns, no sex-specific difference are to be expected regarding mutation status and that either a pure sample of either males or females with premature CAD could be used to estimate prevalence of Familial Hypercholesterolemia in premature CAD patients.

CONCLUSION

DLCN clinical scoring system is not sufficient as a screening tool for Familial Hypercholesterolemia and genetic testing is mandatory for the early diagnosis and management of FH. Genetic testing in our study revealed variants of uncertain significance that require further validation for potential pathogenicity. Larger studies are required to uncover the true prevalence of genetically-confirmed FH in the Egyptian population.

List of Abbreviations

ACMG American College of Medical Genetics

DLCN Dutch Lipid Clinic Network

FH Familial Hypercholestrolemia

PCAD Premature Coronary Artery Disease

NGS Next Generation Sequence

Declarations

Acknowledgments The authors are grateful for the efforts and support of the Egyptian Centre for Research and Regenerative Medicine's management and staff, the Cardiology residents at Kobri El Qobba military complex and for the participants in this study for their contributions.

Disclosure

The authors report that this work was funded by the Egyptian Centre for Research and Regenerative Medicine. and declare no conflicts of interest. The research was not supported by any commercial source; there were no financial relationships with any organizations that might have an interest in the submitted work.

Authors' contributions

HK conceptualized the study. KA and HK designed the study. ZR designed the data collection tool. ZR carried out data collection. ZR performed the data analysis and interpretation. ZR wrote the original draft. HK, ER and TE revised the article before submission.

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