American Journal of Case Reports

e-ISSN 1941-5923
© Am J Case Rep, 2023; 24: e939489
DOI: 10.12659/AJCR.939489

Received: 2023.01.11
Accepted: 2023.02.21
Available online: 2023.03.23
Published: 2023.04.27

Case Series of Genetically Confirmed Index Cases of Familial Hypercholesterolemia in Primary Care

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDEF 1 Aisyah Kamal
BCDEF 1 Johanes Dedi Kanchau
BCDE 1 Nur Syahirah Shahuri
DE 2 Mohamed-Syarif Mohamed-Yassin
DE 1,2 Noorhida Baharudin
DE 1,2,3 Suraya Abdul Razak
DE 2 Siti Fatimah Badlishah-Sham
DE 2,4 Hasidah Abdul-Hamid
DE 5 Aznida Firzah Abdul Aziz
DEF 1,6 Alyaa Al-Khateeb
DEF 1,6 Yung An Chua
DE 1,7 Noor Alicezah Mohd Kasim
DEF 1,6 Siti Hamimah Sheikh Abdul Kadir
DE 1 Hapizah Nawawi
DE 4 Nadeem Qureshi
ADEG 1,2 Anis Safura Ramli

1 Institute of Pathology, Laboratory and Forensic Medicine (I-PPerForM), Universiti Teknologi MARA, Sungai Buloh, Selangor, Malaysia
2 Department of Primary Care Medicine, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh, Selangor, Malaysia
3 Cardio Vascular and Lungs Research Institute (CaVaLRI), Universiti Teknologi MARA, Bandar Puncak Alam, Selangor, Malaysia
4 Centre of Academic Primary Care, University of Nottingham, Nottingham, United Kingdom
5 Department of Family Medicine, Universiti Kebangsaan Malaysia, Cheras, Kuala Lumpur, Malaysia
6 Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh, Selangor, Malaysia
7 Department of Pathology, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh, Selangor, Malaysia

Corresponding Author: Anis Safura Ramli, e-mail: anis014@uitm.edu.my

Financial support: This study was funded by the Newton-Ungku Omar Fund (NUOF): The UK-Malaysia Joint-Partnership Call on Non-Communicable Diseases – Reducing Premature Coronary Artery Disease by Early Identification of Familial Hypercholesterolemia, Grant reference: 100-TNCPI/GOV 16/6/2 (002/2020)-02 and MR/T 017384/1

Conflict of interest: None declared

Case series

Patients: Female, 39-year-old • Female, 57-year-old • Male, 41-year-old

Final Diagnosis: Familial hypercholesterolemia

Symptoms: Asymptomatic

Clinical Procedure: —

Specialty: Family Medicine

Objective: Rare disease

Background: In Malaysia, the prevalence of genetically confirmed heterozygous familial hypercholesterolemia (FH) was reported as 1 in 427. Despite this, FH remains largely underdiagnosed and undertreated in primary care.

Case Reports: In this case series, we report 3 FH cases detected in primary care due to mutations in the low-density lipoprotein receptor (*LDLR*), apolipoprotein-B (*APOB*), and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) genes. The mutations in case 1 (frameshift c.660del pathogenic variant in *LDLR* gene) and case 2 (missense c.10579C>T pathogenic variant in *APOB* gene) were confirmed as pathogenic, while the mutation in case 3 (missense c.277C>T mutation in *PCSK9* gene) may have been benign. In case 1, the patient had the highest LDL-c level, 8.6 mmol/L, and prominent tendon xanthomas. In case 2, the patient had an LDL-c level of 5.7 mmol/L and premature corneal arcus. In case 3, the patient had an LDL-c level of 5.4 mmol/L but had neither of the classical physical findings. Genetic counseling and diagnosis were delivered by primary care physicians. These index cases were initially managed in primary care with statins and therapeutic lifestyle modifications. They were referred to the lipid specialists for up-titration of lipid lowering medications. First-degree relatives were identified and referred for cascade testing.

Conclusions: This case series highlights different phenotypical expressions in patients with 3 different FH genetic mutations. Primary care physicians should play a pivotal role in the detection of FH index cases, genetic testing, management, and cascade screening of family members, in partnership with lipid specialists.

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

Keywords: Atherosclerosis • Coronary Artery Disease • Hyperlipidemia with Familial Hypercholesterolemic Xanthomatosis • Physicians, Primary Care

Full-text PDF: https://www.amjcaserep.com/abstract/index/idArt/939489

Background

Familial hypercholesterolemia (FH) is an inherited genetic disorder with an autosomal dominant mode of inheritance [1]. It is one of the most common monogenic diseases that leads to an elevated risk of premature atherosclerosis, due to its effect on plasma cholesterol levels [2]. FH is commonly caused by mutations in the low-density lipoprotein receptor (*LDLR*) gene, apolipoprotein B (*APOB*) gene, and/or proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene [3], with the most common mutations found in the *LDLR* gene, followed by *APOB* and *PCSK9* genes [4]. Other rare mutations in the LDL receptor adaptor protein 1 (*LDLRAP1*) gene, apolipoprotein E (*APOE*) gene, and ATP-binding cassette subfamily G members 5 and 8 (*ABCG5* and *ABCG8*) genes have been reported to be responsible for the recessive form of FH [5,6]. There are 2 forms of FH: the heterozygous form (HeFH), in which the patients have 1 normal allele and 1 mutated allele; and the homozygous form (HoFH), in which patients have 2 mutated alleles [1].

Clinically, the key characteristics of patients with FH are severely elevated low-density lipoprotein cholesterol (LDL-c), premature corneal arcus (<45 years old), and tendon xanthomas, predominantly in the Achilles tendon and on the extensor tendons of the elbows, hands, knees, and toes [7,8]. FH can be identified and diagnosed using established clinical diagnostic criteria, such as Simon Broome (SB) criteria [9] and Dutch Lipid Clinic Criteria (DLCC) [10]. Genetic testing to confirm the diagnosis can be offered if resources are available [11].

HoFH is rarely reported [12], with a global prevalence estimated at 1 in 160 000 to 1 in 300 000 [7]. HeFH is more common, with a global prevalence estimated at 1 in 200 to 1 in 500 [13]. A recent systematic review and meta-analysis showed that the pooled prevalence of HeFH based on genetic testing or established clinical diagnostic criteria among the general adult population was 1 in 303 [14]. In Malaysia, the prevalence of clinically diagnosed HeFH was estimated to be 1 in 100 [15], while the prevalence of genetically confirmed HeFH was recently reported as 1 in 427 [16]. This is comparable to the prevalence in other countries [14].

If FH is left untreated, men with FH have a 50% risk of developing coronary artery disease (CAD) by the age of 50 years, and women with FH have a 30% risk of developing CAD by the age of 60 [17]. FH remains underdiagnosed and undertreated, especially in the primary care setting [13]. This results in a lost opportunity for preventing premature CAD [13,15]. Therefore, early identification in primary care of patients who are at risk of FH is pivotal. However, the established clinical criteria, such as SB and DLCC, have limited utility for the purposes of case-finding in primary care [18], as they were developed from secondary care registers of FH patients. These diagnostic criteria would require a comprehensive physical examination (tendon xanthoma and arcus cornealis), family history recording, and genetic mutation testing, which are not routinely assessed or are inadequately documented in primary care. As a consequence, researchers in the United Kingdom have developed a new case-finding tool, the Familial Hypercholesterolemia Case Ascertainment Tool (FAMCAT), based on a risk prediction algorithm developed and validated from primary care databases [18,19]. In Malaysia, genetic testing is not routinely available due to limited financial resources as well as a lack of expertise to conduct genetic testing. Therefore, the SB, DLCC, and FAMCAT can be used to identify FH cases in primary care when genetic testing is unavailable.

With or without genetic testing, patients with clinically diagnosed FH are at a very high risk to develop atherosclerotic cardiovascular diseases (ASCVD) and therefore should be treated with high-dose potent statins and therapeutic lifestyle modifications [20,21]. If patients fail to achieve an LDL-c target of <1.8 mmol/L, the current Malaysian clinical practice guideline recommends the combination of high-dose potent statins with either ezetimibe or *PCSK9* inhibitors [22]. Since FH is an inherited genetic condition, its diagnosis should lead to cascade screening of family members to identify those affected [23]. In this case series, we report 3 patients with FH who were clinically diagnosed in primary care and, subsequently, were genetically tested.

Case Reports

Methodology

The 3 patients reported in this case series were participants of a study titled "Reducing Premature Coronary Artery Disease by Early Identification of Familial Hypercholesterolemia: The UK-Malaysia Joint-Partnership Call on Non-Communicable Diseases"

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

(grant reference: 100-TNCPI/GOV 16/6/2 [002/2020]-02 and MR/T 017384/1).

These 3 patients were identified in the primary care setting from September 2020 to April 2021 and were invited to participate. A patient information sheet about the study was given, and written informed consent was obtained upon recruitment into the study. Written informed consent to use the clinical data and digital images was also obtained from all patients in this case series.

Demographic data (age, ethnicity, education level, marital status, household income, and family history) were obtained from the patients. Information on symptoms of angina and peripheral vascular disease (PVD) were collected using the WHO Rose Angina Questionnaire [24] and Edinburgh Claudication Questionnaire [25], respectively. Information on potential secondary causes of hypercholesterolemia, such as chronic kidney disease (CKD), diabetes, and hypothyroidism, were obtained from the patients and verified with their electronic medical records (EMR). Data on current medications and the highest ever recorded fasting serum lipid profiles were obtained from the EMR. Family history variables (elevated cholesterol level, FH, and myocardial infarction) were collected using a validated Family History Questionnaire, which has been translated into the Malay language. Physical examinations to identify tendon xanthomas and corneal arcus were conducted during the consultation.

Using these variables, the SB criteria, DLCC score, and FAMCAT relative risk score was assessed for each patient. They would be clinically diagnosed to have FH if they fulfilled "definite" or "possible" FH by SB criteria [9], DLCC score ≥6 ("definite" or "probable" FH) [10], or FAMCAT algorithm relative risk score >1 (high probability of FH) [26]. The 3 patients were found to fulfill at least 1 of the FH clinical diagnostic criteria. Therefore, they were offered genetic testing. Primary care physicians were trained by the lipid specialists to deliver genetic counseling to the patients prior to genetic testing. Patients were counseled regarding their risk of having FH, the pattern of inheritance, the genetic testing procedure, confirmation of diagnosis after the genetic testing, further management, and the need for cascade screening of first-degree relatives once they are genetically diagnosed. Written informed consent was obtained from the patients.

Approximately 10 mL of venous blood sample was collected from the patients into an ethylene diamine tetra-acetic acid tube at the primary care clinics. These samples were kept in a temperature-controlled container and delivered to the Universiti Teknologi MARA genetic laboratory within 3 h of collection. Once received, genomic DNA was extracted from the whole blood collected using a MasterPure DNA Purification Kit for Blood Version II (Epicentre, Madison, Winsconsin), according to the manufacturer's protocol. The concentration and purity of extracted DNA were determined using a SpectraMax QuickDrop micro-volume spectrophotometer (Molecular Devices, San Jose, CA, USA) followed by a Qubit fluorometer (Thermo Fisher Scientific, Wilmington, DE, USA). The samples were then subjected to targeted next generation sequencing, covering all the exons (including the exon-intron boundaries) and the 5' and 3' untranslated regions of the 4 FH major genes (*LDLR*, *APOB, PCSK9*, and *LDLRAP1*). Library preparation was performed using an AmpliSeq Library PLUS kit (Illumina, San Diego, CA, USA), and enriched samples were sequenced on an ISeq 100 sequencer platform (Illumina, San Diego, CA, USA), according to the manufacturer's instructions. A GRCh37 hg19 human reference assembly was used to map genomic sequencing data using the proprietary BaseSpace Sequence Hub application (Illumina, San Diego, CA, USA). A Germline Variant Caller (Illumina, San Diego, CA, USA) was used to perform variant calling. Only variants with minor allele frequency ≤5% in the 1000 Genomes database were assessed, using in silico web-based software, ClinVar and Leiden Open Variation Database, previous published reports, and clinical data from the 3 cases. The pathogenicity of the variants was agreed based on the American College of Medical Genetics and Genomics guidelines [27], by which variants were classified as pathogenic, likely pathogenic, benign, likely benign, or variants of uncertain significance. Likely pathogenic and pathogenic variants were collectively referred to as pathogenic variants.

Lipid specialists trained the primary care physicians to deliver genetic analysis results to the patients. During consultation, patients who were genetically confirmed to have FH were counseled regarding the nature of the genetic mutations, mode of inheritance, and need for further management, including cascade screening of first-degree relatives. The importance of adherence to lifestyle modification and pharmacotherapy were emphasized. Lipid-lowering treatment for each patient was reviewed and up-titrated to achieve an LDL-c target of <1.8 mmol/L [20,22]. Patients were also supported psychosocially on how to adapt with the condition. They were referred to the Universiti Teknologi MARA lipid specialists for further management and for the cascade screening of first-degree relatives.

Case 1

This patient was a 39-year-old Malay woman, working as a civil servant, who presented to a primary care clinic for routine follow-up for hypercholesterolemia. She was on atorvastatin 20 mg at night and therapeutic lifestyle modification, which included advice on dietary intake and physical activity. This patient was asymptomatic, with no personal history of hypertension, diabetes, or hypothyroidism. The WHO Rose Angina Questionnaire [24] and the Edinburgh Claudication

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

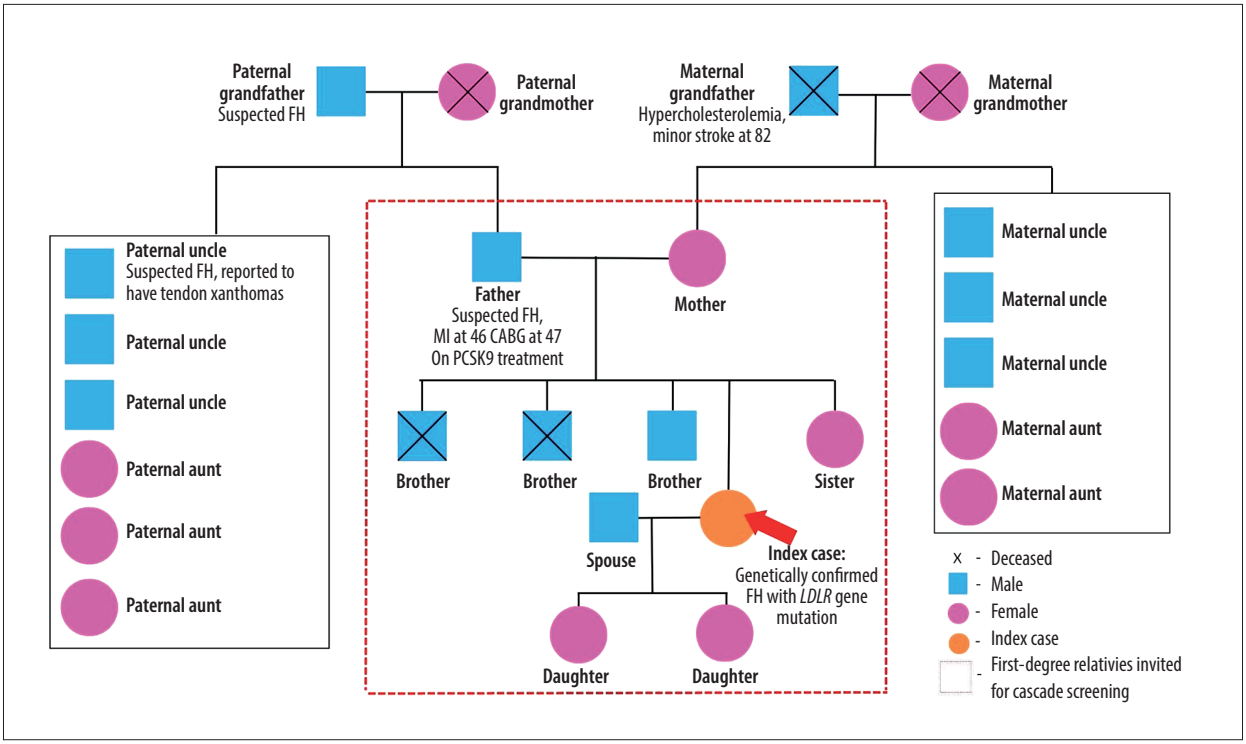

Figure 1. Family pedigree chart for case 1.

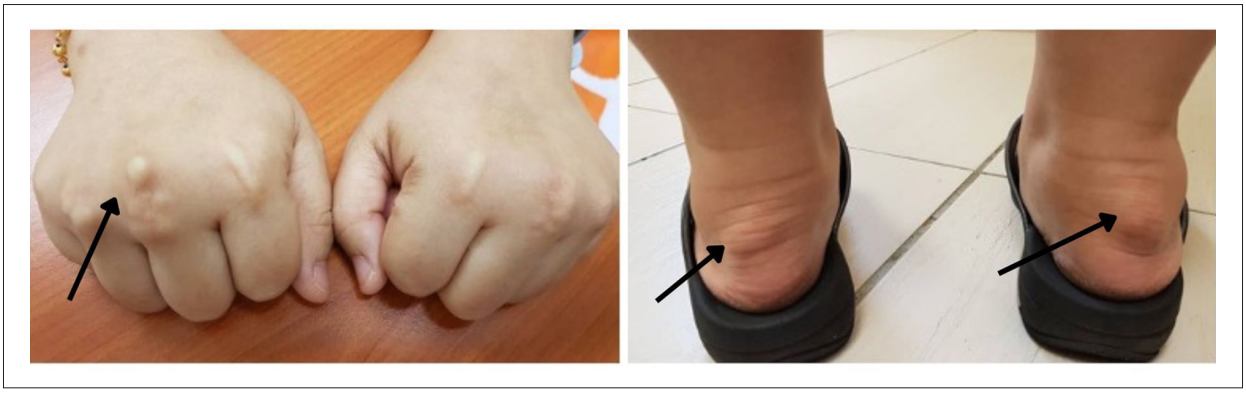

Figure 2. Tendon xanthomas on the metacarpophalangeal joints and Achilles tendon.

Questionnaire [25] were negative. There was also no history of CAD, cerebrovascular disease (CVD), PVD, or CKD. She was a non-smoker and did not drink alcohol.

It was noted that she had a strong family history of elevated cholesterol level and premature CAD, particularly on the paternal side. Her father was suspected to have FH at the age of 42 years old. He had an episode of myocardial infarction (MI) at the age of 46 years old and eventually underwent coronary artery bypass grafting (CABG) 1 year after the MI, at the age of 47 years. He was currently 65 years old and was being treated with a *PCSK9* inhibitor as a lipid-lowering treatment. Her paternal grandfather and uncle were also suspected to have FH and were reported to have tendon xanthomas.

On the maternal side, the family history was uneventful, as her maternal grandfather had a minor stroke at the age of 82 years old. Despite these findings, none of her family members had undergone genetic testing. Her family pedigree chart is shown in **Figure 1**.

On examination, her vital signs were normal. There were tendon xanthomas on her bilateral metacarpophalangeal joints, Achilles tendon, and knees. There was no premature corneal arcus. Other physical examinations, including of the cardiovascular and respiratory systems, were unremarkable. **Figure 2** shows the physical examination findings.

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

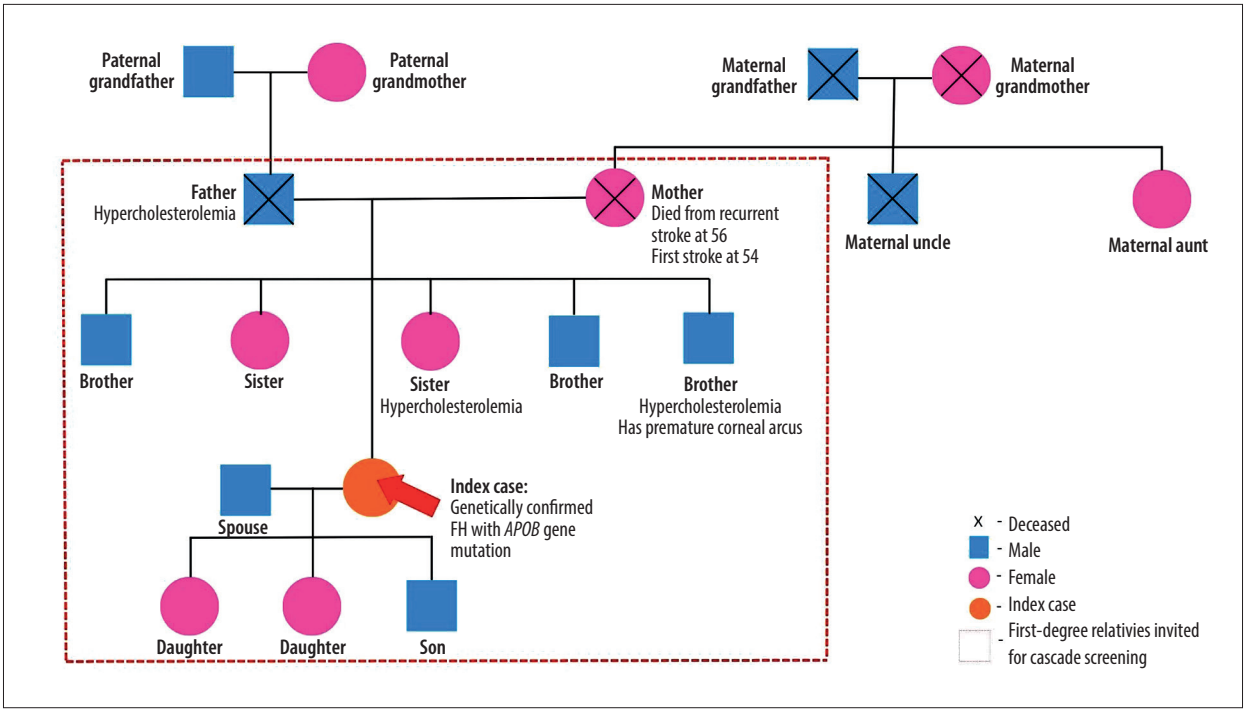

Figure 3. Family pedigree chart for case 2.

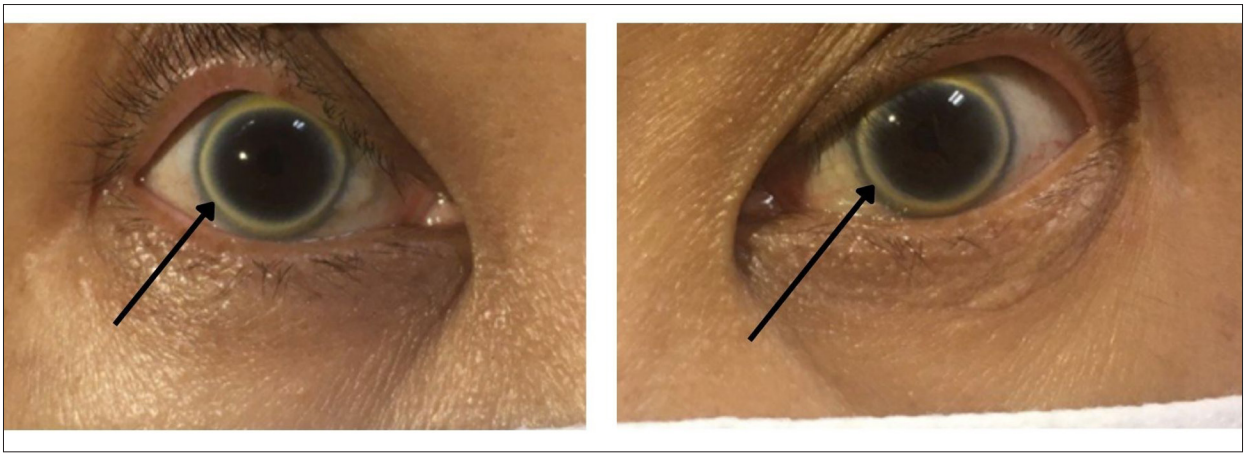

Figure 4. Stage 4 corneal arcus in both eyes.

According to the data extracted from her EMR, the highest cholesterol level was noted in 2019, with an LDL-c of 8.6 mmol/L and a total cholesterol (TC) of 10.4 mmol/L. In terms of clinical diagnosis, this patient fulfilled the SB criteria (definite FH), DLCC (score of 16: definite FH), and FAMCAT (relative risk score of 32.3). Upon targeted next generation sequencing analysis, the patient was found to carry a frameshift c.660del pathogenic variant in the *LDLR* gene (rs875989905) located in exon 4. The type of mutation was frameshift mutation p.Asp221fs. Therefore, this patient was confirmed to have HeFH by genetic testing. Despite pharmacological therapy with statin and therapeutic lifestyle modification, she failed to achieve the LDL-c target of <1.8 mmol/L and was therefore referred to the lipid specialists for further management. Cascade screening of first-degree relatives is currently being conducted by the lipid specialists in order to identify those affected with FH.

Case 2

This patient was an unemployed 57-year-old Chinese woman, diagnosed with hypercholesterolemia at the age of 55 years, and with no other known medical illness. She presented at a primary care clinic for a routine follow-up. This patient was on simvastatin 20 mg at night and received advice on dietary intake and physical activity. During this visit, she was assessed using the WHO Rose Angina Questionnaire [24] and described

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

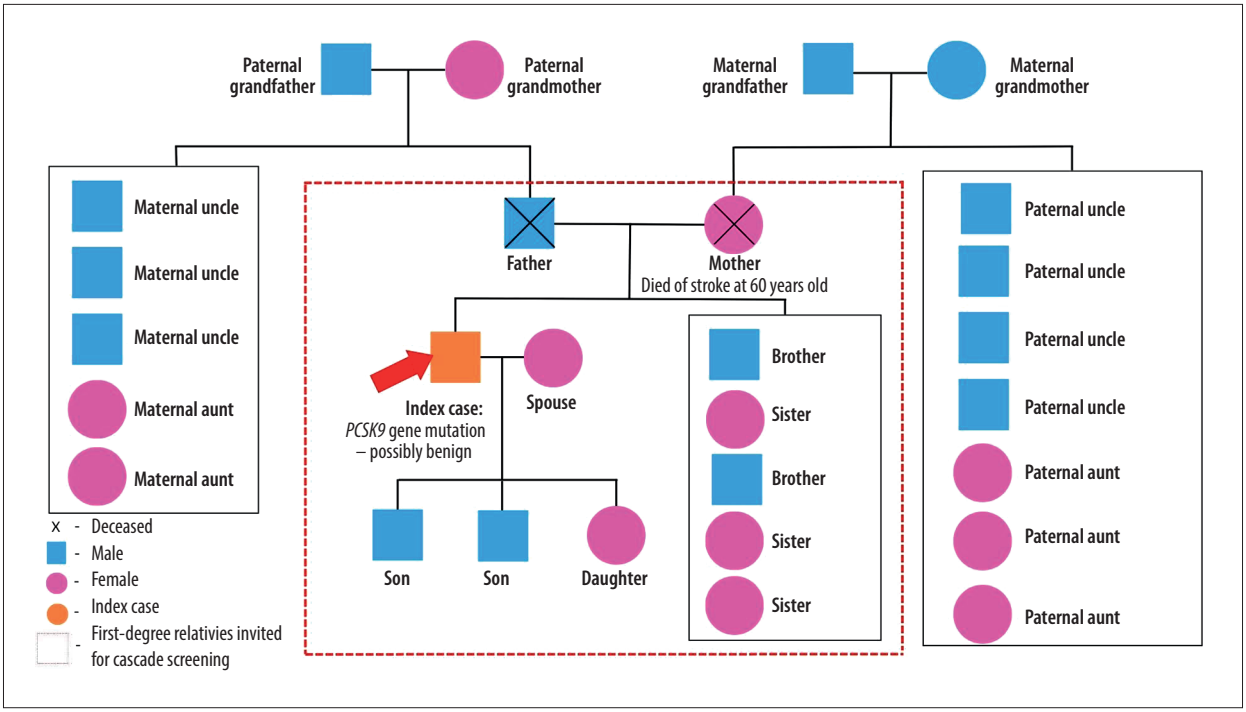

Figure 5. Family pedigree chart for case 3.

having a history of central chest tightness at the age of 55 years, which resolved in less than 5 min. The pain was worse after a meal and was not exacerbated by exertion or physical activity. She never had any chest pain at rest or any chest discomfort that lasted more than 30 min. She did not seek medical attention at the time, and it was not further investigated. She was also assessed using the Edinburgh Claudication Questionnaire [25] and was found to be negative for the presence of PVD symptoms. There was no personal history of secondary causes of hypercholesterolemia, such as diabetes, hypertension, or hypothyroidism. There was no history of CAD, CVD, PVD, or CKD recorded in her EMR. She drank alcohol occasionally but did not smoke cigarettes.

This patient has a significant family history of hypercholesterolemia. Her father, elder sister, younger brother, and younger sister were all on lipid-lowering therapy. She noted that her elder sister had xanthelasma around the eyes and premature corneal arcus in her forties. Her younger brother also had premature corneal arcus in his forties. Her mother died of a recurrent stroke at the age of 56 years old; the first stroke occurred at the age of 54 years. **Figure 3** depicts the patient's family pedigree chart.

On examination, she was well and did not appear to be in pain. Her vital signs were normal. Stage 4 corneal arcus was present in both eyes, as shown in **Figure 4**; she noticed that they started in her thirties. Other physical examinations were unremarkable.

According to the data extracted from her EMR, the highest cholesterol level was noted in 2019, with an LDL-c of 5.7 mmol/L and TC of 8.5 mmol/L. Based on these findings, this patient fulfilled the SB criteria (possible FH), DLCC (score of 14: definite FH), and FAMCAT (relative risk score of 4.4). The genetic analysis of this patient showed a missense c.10579C>T pathogenic variant in the *APOB* gene (rs144467873), located in exon 26. The type of mutation was a missense mutation, p.Arg3527Trp, which confirmed the diagnosis of HeFH in this patient.

This patient was immediately referred to the family medicine specialist at the primary care clinic for further assessment of her chest pain. Her resting electrocardiogram was normal, and she was given a follow-up appointment with the family medicine specialist for further monitoring of her chest pain. Despite pharmacological therapy and therapeutic lifestyle modification, her LDL-c and TC levels remained high and she failed to achieve the LDL-c target of <1.8 mmol/L. Therefore, she was also referred to the lipid specialists for further management. Cascade screening of first-degree relatives is currently being conducted by the lipid specialists in order to identify those affected with FH.

Case 3

This patient was a 41-year-old Malay man with underlying hypertension. He was working as a government official. He came to a primary care clinic for a routine follow-up. He was asymptomatic, with no subclinical evidence of ASCVD. There was no personal history of hypercholesterolemia, diabetes, or

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

hypothyroidism. The WHO Rose Angina Questionnaire [24] and the Edinburgh Claudication Questionnaire [25] were negative. There was no history of CAD, CVD, PVD, or CKD recorded in the EMR. This patient had stopped smoking in 2016. He had smoked 10 pack years of cigarettes since he was 18 years old. His mother had a stroke at 60 years of age. There were no other family members with history of hypercholesterolemia, CAD, CVD, or PVD. His family pedigree chart is shown in **Figure 5**.

On examination, his vital signs were normal. There was no tendon xanthoma or premature corneal arcus. Other physical examinations, including the cardiovascular and respiratory systems, were unremarkable.

According to the data extracted from his EMR, the highest cholesterol level was noted in 2020, with an LDL-c of 5.4 mmol/L and a TC of 7.3 mmol/L. This patient fulfilled the SB criteria (possible FH) but did not fulfill the DLCC (score of 4) and FAMCAT (relative risk score of 0.6). The genetic analysis of this patient showed a missense c.277C>T mutation in the *PCSK9* gene (rs151193009), which was located within exon 2. There are conflicting findings whether this particular mutation is pathogenic, a variant of uncertain significance, or benign [28-31].

He was initially managed in primary care with simvastatin 10 mg at night and therapeutic lifestyle modification, which included advice on dietary intake and physical activity. However, he failed to achieve the LDL-c target of <1.8 mmol/L and was therefore referred to the lipid specialists for further management. Cascade screening of first-degree relatives is currently being conducted by the lipid specialists.

Table 1 shows a summary of all 3 patients selected for this case series, who were identified in primary care and had subsequently been genetically tested.

Discussion

This case series is the first to report genetically confirmed HeFH in the Malaysian primary care setting. We presented 3 cases of patients aged 39 to 57 years old with *LDLR*, *APOB*, and *PCSK9* gene mutations. In case 1, the patient was confirmed to have a pathogenic *LDLR* gene frameshift mutation, while the patient in case 2 was confirmed to have a pathogenic *APOB* gene missense mutation. In case 3, the patient had a *PCSK9* missense c.277C>T mutation on p.Arg93Cys (rs151193009). There are conflicting findings whether this particular mutation is pathogenic, a variant of uncertain significance, or benign [28-31].

In Asian populations, including Malaysian, the most common FH mutation that has been described is of the *LDLR* gene [32-34], followed by *APOB* and *PCSK9* [33]. It was previously reported that only 10% to 15% of FH cases in Asia was attributable to *APOB* gene mutations [33], which is more common in Taiwan (9.2%) and China (10.9%) [35]. In contrast, mutations of the *PCSK9* gene were more common in Japan, with a frequency of 7.8% [36]. *PCSK9* mutation was found to be rare in China and Korea, and none was reported in Taiwan [35]. In Malaysia, published literature that described the prevalence of *APOB* and *PCSK9* gene mutations is limited [32].

With regards to LDL-c levels, the patients presented in this case series had high levels of LDL-c, ranging from 5.4 to 8.6 mmol/L, which is 3 to 5 times higher than the target value of 1.8 mmol/L for individuals at very high cardiovascular risk [22]. This finding is similar to that of a Canadian study involving 313 genetically confirmed HeFH patients, in which 42% of the patients had LDL-c levels between 5.0 and 6.0 mmol/L, and 88% of the patients had an LDL-c level above 8.0 mmol/L [37]. It was observed that the patient in case 1, who was confirmed to have an *LDLR* gene mutation, had the highest LDL-c level at 8.6 mmol/L, compared with case 2 with an *APOB* gene mutation and level of 5.7 mmol/L and case 3 with a *PCSK9* gene mutation and level of 5.4 mmol/L. Typically, FH patients with mutations in the *LDLR* gene show the highest LDL-c levels compared with patients with mutations in other FH candidate genes [38,39]. The genetic defects in the *LDLR* gene causes LDL-c to be removed from the circulation at half the normal rate, causing an accumulation of lipoprotein level in the blood at 2-fold above normal [40]. The c.660delC pathogenic variants results from a deletion of 1 nucleotide at position 660, causing a translational frameshift change with a predicted stop codon (p.Asp221fs). This mutation has been reported among FH cohorts from various ethnic groups [41,42]. This *LDLR* pathogenic variant is expected to result in premature protein truncation or nonsense-mediated mRNA decay, with subsequent loss of protein function [43].

With regards to the p.Arg3527Trp pathogenic variant that is located in the *APOB* gene, our finding is consistent with the findings of other studies which observed that among patients of the same age, body mass index, and sex, those with an *APOB* gene defect had elevated LDL-c values but relatively lower values than those with a pathogenic *LDLR* gene mutation [44,45]. Additionally, for p.Arg3527Trp, the arginine at codon 3527 is replaced by tryptophan, an amino acid with different properties. Adding to that, it is located in an *APOB* region that is vital for protein folding and stability, suggesting that this variant can affect protein function. Based on this evidence, this variant is classified as pathogenic for FH.

The *PCSK9* missense c.277C>T mutation on p.Arg93Cys found in case 3 has never been previously reported in the Malaysian population. This mutation was described as a pathogenic variant among a Chinese population with premature acute MI [28]. However, 3 other studies showed that this loss-of-function

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

Table 1. Summary of the genetic mutations of patients presented in this case series.

Details	Case 1	Case 2	Case 3
Age (years)	39	57	41
Sex	Female	Female	Male
Personal history			
Premature coronary artery disease	No	No	No
ROSE Angina Questionnaire results	Negative	Positive	Negative
Premature cerebrovascular disease	No	No	No
Edinburgh Claudication Questionnaire results	Negative	Negative	Negative
Chronic kidney disease	No	No	No
Diabetes	No	No	No
Hypothyroidism	No	No	No
Family history			
Premature coronary heart disease (male <55 y; female <60 y)	Yes Father had MI at 46 years of age and CABG at 47 years old	No	No
Premature cerebrovascular or peripheral vascular disease (male <55 y; female <60 y)	No Maternal grandfather had a minor stroke at 82 years of age	Yes Mother had a stroke at 54 years old, deceased from a recurring stroke at 56 years of age	Yes Mother had a stroke at 60 years old
Hypercholesterolemia	Yes Father – on PCSK9 inhibitor; paternal grandfather; paternal uncle; maternal grandfather	Yes Father and siblings	No
First degree relatives with corneal arcus	No	Yes Elder sister & younger brother in their 40s	No
First degree relatives with tendon xanthomas	Yes Father, paternal uncle, paternal grandfather	No	No
Physical examination			
Xanthomas	Yes Knuckles, Achilles tendon, knees	No	No
Premature corneal arcus (<45 years old)	No	Yes Patient noticed since her 30 s	No
Medication			
Current medication	Atorvastatin 20 mg	Simvastatin 20 mg	Simvastatin 10 mg

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

Table 1 continued. Summary of the genetic mutations of patients presented in this case series.

Details	Case 1	Case 2	Case 3
Fasting serum lipid			
The highest TC ever recorded	10.4	8.5	7.3
The highest LDL-c ever recorded	8.6	5.7	5.4
Triglycerides	1.2	0.9	2.1
HDL-c	1.3	2.4	0.9
Clinical diagnostic criteria			
Simon Broome criteria	Definite	Possible	Possible
DLCC score	16	14	4
FAMCAT relative risk score	32.3	4.4	0.6
Mutation			
Gene	*LDLR*	*APOB*	*PCSK9*
Exon	4	26	2
Nucleotide change	c.660del	c.10579C>T	c.277C>T
Chromosome position	chr19-11216238-GC-G	chr2-21229161-G-A	chr1-55509585-C-T
rs number	rs875989905	rs144467873	rs151193009
Amino acid change	p.Asp221fs	p.Arg3527Trp	p.Arg93Cys
Type of mutation	Frameshift (deletion)	Missense	Missense
Pathogenicity of variants based on ACMG Guidelines	Pathogenic	Pathogenic	Likely benign

CKD – chronic kidney disease; TC – total cholesterol; LDL-c – low-density lipo-protein cholesterol; HDL-c – high-density lipoprotein cholesterol; DLCC – Dutch Lipid Clinic Criteria; FAMCAT – familial hypercholesterolemia case ascertainment identification tool; ACMG – American College of Medical Genetics and Genomics.

mutation was significantly associated with lower LDL-c levels and a reduced risk of premature MI of approximately 60% [29]. A study in Japan also showed the LDL-c levels were significantly lower in patients harboring the *PCSK9* gene mutation than those with the *LDLR* gene mutation [36]. In case 3, the patient fulfilled the SB criteria as "possible FH", because the highest LDL-c level was 5.4 mmol/L, and his mother had a stroke at 60 years old. He did not have any subclinical evidence of ASCVD. Therefore, the *PCSK9* missense c.277C>T mutation in this patient may not have been pathogenic. However, he failed to achieve the LDL-c treatment target of <1.8 mmol/L. This patient was still referred to the lipid specialists for up-titration of lipid lowering treatment and cascade screening of first-degree relatives.

A higher LDL-c level in the blood has been associated with a more severe expression of phenotypes [46]. Phenotypically, we found that the patient in case 1, who was confirmed to have *LDLR* deletion gene mutation, presented with a more severe phenotype, namely tendon xanthomas, compared with the patient in case 2 with *APOB* pathogenic variants. These findings are parallel to a previous local study involving 164 FH patients, in which those with a frameshift mutation of the *LDLR* gene showed a more severe phenotype expression and higher LDL-c level [44]. Another case of HeFH in Japan reported similar findings, in which a female patient with an *LDLR* gene mutation showed systemic xanthomatosis, including thickening of the Achilles tendon and an elevated LDL-c level of 7.6 mmol/L [47]. Regarding the *APOB* gene mutation, although the expression is less severe phenotypically compared with that of carriers of *LDLR* gene defects, the *APOB* gene mutation is still more strongly associated with a higher risk of CVD and TC: HDL ratio than in those without mutation [44].

FH is still currently underdiagnosed in Malaysia, with a detection rate estimated at 0.5% [34]. Primary care physicians should have a high index of suspicion in patients presenting with LDL-c levels of >4.9 mmol/L [22,23]. In this case series, primary care physicians were trained on how to perform a thorough

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

physical examination and to be familiar with the appropriate clinical diagnostic tools, such as the SB, DLCC, and FAMCAT case-finding tool. Referral for genetic testing should be considered if the patient fulfills the clinical diagnostic criteria and if genetic testing is routinely available, as this is the criterion standard to diagnose FH [48]. As with many developing countries, genetic testing is not routinely available in Malaysia owing to limited financial resources. However, in this case series, patients who fulfilled at least 1 of the FH clinical diagnostic criteria were offered genetic testing, which was funded by the study. Primary care physicians were trained by the lipid specialists to deliver genetic counseling to the patients prior to genetic testing. The counseling included informing the patients of their risk of having FH, the pattern of inheritance, genetic testing procedure, confirmation of diagnosis after the genetic testing, further management, and need for cascade screening of first-degree relatives once they are genetically diagnosed.

Primary care physicians were also trained to deliver genetic analysis results to the patients. In this cases series, once the patients were confirmed to have FH, they were counseled regarding the nature of the genetic mutations and mode of inheritance. These patients' cases were initially managed in primary care with statins and therapeutic lifestyle modification, which included advice on dietary intake and physical activity. Patients with FH are recommended to receive individualized advice about diet, physical activity, and healthy weight maintenance from healthcare providers to reduce their cardiovascular risks [49-51]. With regards to the pharmacological management for patients with FH, the current Malaysian clinical practice guideline recommends the combination of high-dose potent statins with either ezetimibe or *PCSK9* inhibitors for those who fail to achieve the LDL-c target of <1.8 mmol/L [22]. However, awareness of FH is lacking among primary care physicians [13,52,53], which may explain the low-potency statins being prescribed for patients in this case series. While access to ezetimibe and a *PCSK9* inhibitor are limited for primary care physicians, higher-potency statins, such as atorvastatin, are widely available in primary care and should be used appropriately. In this case series, these patients were referred to the lipid specialists for up-titration of lipid-lowering medication, as they failed to achieve the LDL-c treatment target.

Apart from pharmacological treatment with lipid-lowering medications, cascade screening of first-degree relatives should also be conducted to allow early detection and management of this autosomal dominant disorder. Cascade screening is a cost-effective method to identify additional cases of FH through systematic family tracing [54]. In this case series, primary care physicians also played a significant role in identifying first-degree relatives to be referred for cascade testing. Early detection of FH will reduce the average age at which family members are diagnosed with FH and increase the percentage of them receiving pharmacological treatment [54]. In addition to the above measures, long-term follow-up by both a primary care physician and lipid specialist is vital to ensure patients with FH achieve the LDL-c target, hence preventing premature ASCVD. The need to integrate the pivotal role of primary care physicians with that of the lipid specialists' service to enhance the detection and management of FH in the community is well recognized [55]. There is extensive evidence to show that this approach is clinically and financially effective [56-58].

Additionally, accurate national registries on the prevalence of FH would assist in increasing the awareness of this condition among clinicians [59]. In Malaysia, a national level FH registry is currently being established [60]. Clinical practice guidelines on the management of FH in Malaysia should also be established to improve early detection and treatment of FH, especially in primary care, which serves as the front-line of the healthcare setting. It is hoped that these systematic and widespread efforts will improve the prevention of premature ASCVD in this population.

Conclusions

In summary, we reported 3 cases of patients with mutations of *LDLR*, *APOB*, and *PCSK9* genes seen in primary care. Consistent with the literature, the patient with the *LDLR* gene mutation in this case series showed a higher LDL-c level and a more prominent phenotype compared with the other 2 patients. This case series highlights the differences in phenotypical expression in patients with genetic mutations of 3 different genes. Primary care physicians should have a high index of suspicion in patients presenting with LDL-c levels of >4.9 mmol/L. These patients should be thoroughly assessed and clinically diagnosed with established clinical diagnostic criteria, such as the SB and DLCC, as well as the FAMCAT case-finding tool. Referral for genetic testing should be considered if the testing is routinely available. With or without genetic testing, clinically diagnosed patients with FH should be treated accordingly, with combinations of high-dose potent lipid-lowering medications, and cascade screening of first-degree relatives should be performed. Primary care physicians should play a pivotal role in the detection of FH index cases, genetic testing, management, and cascade screening of family members in partnership with the lipid specialists. Establishment of a national FH registry and clinical practice guideline are also important to improve awareness and guide the treatment of patients with FH and subsequently reduce morbidity and mortality from premature ASCVD.

Acknowledgment

The study protocol was approved by the respective research ethics committees in Malaysia i.e. the UiTM Research Ethics Committee [(REC/03/2020) (FB/48)] and the Medical Research

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

Ethics Committee of the Ministry of Health Malaysia [NMRR-20-272-52797 (IIR)]. We also would like to thank the Director General of Health Malaysia for his permission to publish this article. Our gratitude also goes to the research assistants who have worked on this project and to all the site investigators and nurses at the Ministry of Health primary care clinics who have rendered their assistance and support towards this study.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

References:

1. Pejic RN. Familial hypercholesterolemia. Ochsner J. 2014;14:669-72
2. Vrablik M, Tichý L, Freiberger T, et al. Genetics of familial hypercholesterolemia: New insights. Front Genet. 2020;11:574474
3. Doi T, Hori M, Harada-Shiba M, et al. Patients with LDLR and PCSK9 gene variants experienced higher incidence of cardiovascular outcomes in heterozygous familial hypercholesterolemia. J Am Heart Assoc. 2021;10:e018263
4. Truong PK, Lao TD, Le THA. The major molecular causes of familial hypercholesterolemia. Asian J Pharma Res Health Care. 201810:60-68
5. Garcia CK, Wilund K, Arca M, et al. Autosomal recessive hypercholesterolemia caused by mutations in a putative LDL receptor adaptor protein. Science. 2001;292:1394-98
6. Asselbergs FW, Guo Y, van Iperen EP, at al. Large-scale gene-centric meta-analysis across 32 studies identifies multiple lipid loci. Am J Hum Genet. 2012;91:823-38
7. Alnouri F, Al-Allaf FA, Athar M, et al. Xanthomas can be misdiagnosed and mistreated in homozygous familial hypercholesterolemia patients: A call for increased awareness among dermatologists and health care practitioners. Glob Heart. 2020;15:19
8. Bouhairie, VE, Goldberg, AC. Familial hypercholesterolemia. Cardiol Clin. 2015;33:169-79
9. Mortality in treated heterozygous familial hypercholesterolaemia: Implications for clinical management [Scientific Steering Committee on behalf of the Simon Broome Register Group]. Atherosclerosis. 1999;142:105-12
10. Watts GF, Sullivan DR, Poplawski N, et al. Familial hypercholesterolaemia: A model of care for Australasia. Atheroscler Suppl. 2011;12:221-63
11. Qureshi N, Akyea RK, Dutton B, et al. Case-finding and genetic testing for familial hypercholesterolaemia in primary care. Heart. 2021;107:1956-61
12. Alicezah MK, Razali R, Rahman T, et al. Homozygous familial hypercholesterolemia. Malays J Pathol. 2014;36:131-17
13. Nordestgaard BG, Chapman MJ, Humphries SE, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: Guidance for clinicians to prevent coronary heart disease: Consensus statement of the European Atherosclerosis Society [published erratum appears in Eur Heart J. 2020;41:4517]. Eur Heart J. 2013;34:3478-90a
14. Hu P, Dharmayat KI, Stevens CAT, et al. Prevalence of familial hypercholesterolemia among the general population and patients with atherosclerotic cardiovascular disease: A systematic review and meta-analysis. Circulation. 2020;22:1742-59
15. Vallejo-Vaz AJ, De Marco M, Stevens CAT, et al. Overview of the current status of familial hypercholesterolaemia care in over 60 countries – The EAS Familial Hypercholesterolaemia Studies Collaboration (FHSC). Atherosclerosis. 2018;277:234-55
16. Razman AZ, Chua YA, Mohd Kasim NA, et al. Genetic spectrum of familial hypercholesterolaemia in the malaysian community: Identification of pathogenic gene variants using targeted next-generation sequencing. Int J Mol Sci. 2022;23:14971
17. Goldberg AC, Hopkins PN, Toth, et al. Familial hypercholesterolemia: screening, diagnosis and management of pediatric and adult patients: Clinical guidance from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. J Clin Lipidol. 2011;5(3 Suppl.):S1-8
18. Qureshi N, Weng S, Tranter J, et al. Feasibility of improving identification of familial hypercholesterolaemia in general practice: Intervention development study [published erratum appears in BMJ Open. 2016;: e011734corr1]. BMJ Open. 2016;5:e011734
19. Weng SF, Kai J, Neil HA, et al. Improving identification of familial hypercholesterolaemia in primary care: Derivation and validation of the familial hypercholesterolaemia case ascertainment tool (FAMCAT). Atherosclerosis. 2015;2:336-43
20. National Institute for Health and Clinical Excellence (NICE). Identification and Management of Familial Hypercholesterolemia. United Kingdom: NICE Clinical Guideline; 2019
21. Gidding SS, Champagne MA, de Ferranti SD, et al. The agenda for familial hypercholesterolemia: A scientific statement from the American Heart Association. Circulation. 2015;22:2167-92
22. Ministry of Health (MOH) Malaysia. 5th Edition of Clinical Practice Guidelines: Management of Dyslipidaemia. 2017;20-21
23. Kasim NAM, Chua YA, Nawawi H. Familial hypercholesterolaemia: An updated overall management. J Clin Health Sci. 2020;5:19-38
24. Rahman MA, Spurrier N, Mahmood MA, et al. Rose Angina Questionnaire: Validation with cardiologists' diagnoses to detect coronary heart disease in Bangladesh. Indian Heart J. 2013;65:30-39
25. Boylan L, Nesbitt C, Wilson L, et al. Reliability of the Edinburgh Claudication Questionnaire for identifying symptomatic PAD in general practice. Angiology. 2021;72:474-79
26. Weng S, Kai J, Akyea R, Qureshi N. Detection of familial hypercholesterolaemia: external validation of the FAMCAT clinical case-finding algorithm to identify patients in primary care [published erratum appears in Lancet Public Health. 2019;4(7):e325]. Lancet Public Health. 2019;4:e256-e64
27. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-24
28. Lee C, Cui Y, Song J, et al. Effects of familial hypercholesterolemia-associated genes on the phenotype of premature myocardial infarction. Lipids Health Dis. 2019;18:95
29. Yang L, Pu T, Zhang Y, et al. The R93C variant of PCSK9 reduces the risk of premature MI in a Chinese Han population. Front Genet. 2022;13:875269
30. Hsu LA, Teng MS, Wu S, et al. Common and rare PCSK9 variants associated with low-density lipoprotein cholesterol levels and the risk of diabetes mellitus: A Mendelian randomization study. Int J Mol Sc. 2022;18:10418
31. Yu H, Pu T, Xu M, Gao W. P808 Association between genetic variants in PCSK9/APOB/LDLR and premature myocardial infarction in Han Chinese. Eur Heart J. 2018; 39(Suppl. 1):ehy564-P808
32. Al-Khateeb A, Al-Talib H. Genetic researches among Malaysian familial hypercholesterolaemic population. JUMMEC. 2016;19:1-11
33. Jackson CL, Zordok M, Kullo IJ. Familial hypercholesterolemia in Southeast and East Asia. Am J Prev Cardiol. 2021;6:100157
34. Chua YA, Razman AZ, Ramli AS, et al. Familial hypercholesterolaemia in the Malaysian community: Prevalence, under-detection and under-treatment. J Atheroscler Thromb. 2021;28:1095-107
35. Huang CC, Charng MJ. Genetic diagnosis of familial hypercholesterolemia in Asia. Front Genet. 2020;11:833
36. Hori M, Ohta N, Takahashi A, et al. Impact of LDLR and PCSK9 pathogenic variants in Japanese heterozygous familial hypercholesterolemia patients. Atherosclerosis. 2019;289:101-8
37. Wang J, Dron JS, Ban MR, et al. Polygenic versus monogenic causes of hypercholesterolemia ascertained clinically. Arterioscler Thromb Vasc Biol. 2016;36:2439-45
38. Fahed AC, Nemer GM. Familial hypercholesterolemia: The lipids or the genes? Nutr Metab (Lond). 2011;8:23

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

39. Mabuchi H, Nohara A, Noguchi T, et al. Genotypic and phenotypic features in homozygous familial hypercholesterolemia caused by proprotein convertase subtilisin/kexin type 9 (PCSK9) gain-of-function mutation. Atherosclerosis. 2014;236:54-61
40. Goldstein JL, Brown MS. Regulation of low-density lipoprotein receptors: Implications for pathogenesis and therapy of hypercholesterolemia and atherosclerosis. Circulation. 1987;76:504-7
41. Bamimore MA, Zaid A, Banerjee Y, et al. Familial hypercholesterolemia mutations in the Middle Eastern and North African region: A need for a national registry. J Clin Lipidol. 2015;9:187-94
42. Hu M, Hooper AJ, Bockxmeer FM, et al. Management of familial hypercholesterolemia in Hong Kong. J Atheroscler Thromb. 2016;23:520-31
43. National Center for Biotechnology Information. ClinVar; [VCV000226330.13]. [cited 2022 Dec 28]. Available from: https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000226330.13
44. Al-Khateeb A, Al-Talib H, Mohamed MS, et al. Phenotype-genotype analyses of clinically diagnosed Malaysian familial hypercholestrolemic patients. Adv Clin Exp Med. 2013;22:57-67
45. Ejarque I, Real JT, Martinez-Hervas S, et al. Evaluation of clinical diagnosis criteria of familial ligand defective apoB 100 and lipoprotein phenotype comparison between LDL receptor gene mutations affecting ligand-binding domain and the R3500Q mutation of the apoB gene in patients from a South European population. Transl Res. 2008;151:162-67
46. Al-Khateeb AR, Mohd MS, Yusof Z, Zilfalil BA. Molecular description of familial defective APOB-100 in Malaysia. Biochem Genet. 2013;51:811-23
47. Nishikawa T, Tada H, Nakagawa-Kamiya, T, et al. A case with familial hypercholesterolemia complicated with severe systemic atherosclerosis intensively treated for more than 30 years. J Cardiol Cases. 2020;22:216-20
48. Futema M, Taylor-Beadling A, Williams M, Humphries SE. Genetic testing for familial hypercholesterolemia-past, present, and future. J Lipid Res. 2021;62:100139
49. Kinnear FJ, Hamilton-Shield JP, Stensel DJ, et al. Nutrition and physical activity intervention for families with familial hypercholesterolaemia: Protocol for a pilot randomised controlled feasibility study. Pilot Feasibility Stud. 2020;6:42
50. Watts GF, Gidding S, Wierzbicki AS, et al. Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation. Int J Cardiol. 2014;171:309-25
51. Catapano AL, Graham I, De Backer G, et al. 2016 ESC/EAS Guidelines for the management of dyslipidaemias. Eur Heart J. 2016;37:2999-3058
52. Azraii AB, Ramli AS, Ismail Z, et al. Knowledge, awareness and practice regarding familial hypercholesterolaemia among primary care physicians in Malaysia: The importance of professional training. Atherosclerosis. 2018;277:508-16
53. Batais MA, Almigbal TH, Bin Abdulhak AA, et al. Assessment of physicians' awareness and knowledge of familial hypercholesterolemia in Saudi Arabia: Is there a gap? PLoS One. 2017;12:e0183494
54. Knowles JW, Rader DJ, Khoury MJ. Cascade screening for familial hypercholesterolemia and the use of genetic testing. JAMA. 2017;318:381-82
55. Arnold-Reed DE, Brett T, Troeung L, et al. Detection and management of familial hypercholesterolaemia in primary care in Australia: Protocol for a pragmatic cluster intervention study with pre-post intervention comparisons. BMJ Open. 2017;10:e017539
56. Watts GF, Juniper A, van Bockxmeer F, et al. Familial hypercholesterolaemia: A review with emphasis on evidence for treatment, new models of care and health economic evaluations. Int J Evid Based Healthc. 2012;10:211-21
57. Ademi Z, Watts GF, Pang J, et al. Cascade screening based on genetic testing is cost-effective: Evidence for the implementation of models of care for familial hypercholesterolemia. J Clin Lipidol. 2014;8:390-400
58. Nherera L, Marks D, Minhas R, et al. Probabilistic cost-effectiveness analysis of cascade screening for familial hypercholesterolaemia using alternative diagnostic and identification strategies. Heart. 2011;97:1175-81
59. Amerizadeh A, Javanmard SH, Sarrafzadegan N, Vaseghi G. Familial hypercholesterolemia (FH) registry worldwide: A systematic review. Curr Probl Cardiol. 2022;47:100999
60. Firus Khan AY, Ramli AS, Abdul Razak S, et al. The Malaysian HEalth and WellBeing AssessmenT(MyHEBAT) study protocol: An initiation of a national registry for extended cardiovascular risk evaluation in the community. Int J Environ Res Public Health. 2022;19:11789

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]