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CASE REPORT

Homozygous familial hypercholesterolemia

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Abstract

We report a rare case of homozygous familial hypercholesterolemia (HoFH), a 22-year-old Malay woman who presented initially with minor soft tissue injury due to a cycling accident. She was then incidentally found to have severe xanthelasma and hypercholesterolemia (serum TC 15.3 mmol/L and LDL-C 13.9 mmol/L). She was referred to the Specialized Lipid Clinic and was diagnosed with familial hypercholesterolemia (FH) based on the Simon Broome (SB) diagnostic criteria. There was a family history of premature coronary heart disease (CHD) in that three siblings had sudden cardiac death, and of consanguineous marriage in that her parents are cousins. DNA screening of LDLR and APOB genes was done by Polymerase Chain Reaction (PCR), followed by Denaturing High Performance Liquid Chromatography (DHPLC). Homozygous mutation C255S in Exon 5 of her LDLR gene was found. There was no mutation was found in Exon 26 and Exon 29 of the APOB gene. This report is to emphasize the importance of identifying patients with FH and cascade screening through established diagnostic criteria and genetic studies in order to ensure early detection and early treatment intervention to minimize the risk of developing CHD and related complications.

Keywords: familial hypercholesterolemia, low-density lipoprotein cholesterol (LDL-C), lipid stigmata, premature CHD

INTRODUCTION

Familial hypercholesterolemia (FH), is the most common and severe monogenic form of hypercholesterolemia. It is an autosomal co-dominant disease characterized by an increased plasma low density lipoprotein (LDL)-cholesterol (LDL-C) concentration and premature coronary heart disease (CHD). The clinical phenotype depends on the gene involved and severity of the mutation (or mutations) present. Patients with homozygous or compound heterozygous FH have severe hypercholesterolemia (LDL-C>13 mmol/L) due to a gene dosing effect and without treatment have accelerated atherosclerotic CHD from birth, and frequently die of CHD before the age of 30 years. Cholesterol-lowering therapies have been shown to reduce both mortality and major adverse cardiovascular events in individuals with FH. Lipoprotein apheresis concomitant with lipid-lowering therapy is the treatment of choice for homozygous FH (HoFH). It is estimated that 5 to 10 percent of premature CHD patients suffered from FH.[1] HoFH is rare, occurring in 1:1,000,000 live births, in contrast to its heterozygous form, which has a prevalence of 1:500.[2] With a population of 29 million in Malaysia, it is estimated that 58,000 individuals may be affected and the majority of them are still undiagnosed.[3] In this study, we report a case of HoFH due to previously described LDLR mutation.

CASE REPORT

This is a case of a 22-year-old Malay female who was noted to have marked bilateral xanthelasma (Figure 1). She was identified by a medical officer who attended to her for a minor wound over her leg due to a cycling accident. She had a butterfly-like lesion around her eyes since childhood and a few members of the family also had the same condition.

She had no complains of chest pain, palpitations, shortness of breath and has good

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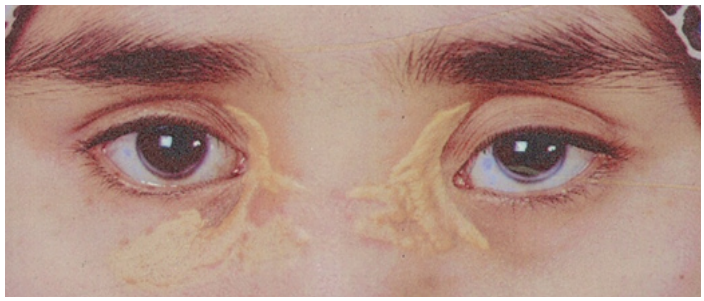


FIG. 1: Xanthelasma. Marked yellow plaques near the inner canthus of the eyelids with bilateral corneal arcus.

effort tolerance. She had neither polyuria, nor polydipsia. No history of lethargy, cold intolerance or menstrual disturbance was observed.

Three of her siblings died at a very young age, which she claimed were due to "heart attacks". There is marital consanguinity in the family in that both parents are cousins. She is single, a non-smoker and claimed to practice a healthy lifestyle.

On examination, she was thin, with a BMI of 18.2, waist circumference of 0.59 m and waist hip ratio of 0.68. Pulse rate was normal and she was normotensive. There was corneal arcus grade 4 on the left side and grade 3 on the right side (Figure 1). Apart from gross xanthelasma on both eyelids, there were digital xanthoma and bilateral Achilles tendon xanthoma. Other systems were unremarkable. Her baseline lipid profile showed markedly elevated total cholesterol (TC) of 15.3 mmol/l, LDL-C of 13.9 mmol/L, HDL-C of 1.6 mmol/l and triglyceride of 1.1 mmol/L. Her thyroid profile, renal profile and fasting plasma glucose were normal. Her baseline ALT and creatinine kinase (CK) were within normal range.

Family screening was performed. The lipid profile of family members was analyzed and several of them showed hypercholesterolemia (Table 1, Figure 2). The diagnosis of FH was made based on Simon Broome Register criteria.[4] Her stress test was negative with Bruce protocol achieved up to Stage 4 (53 seconds). Echocardiogram showed good left ventricular function and normal echocardiographic finding. The carotid intima media thickness (cIMT) of the common carotid artery (CCA), the carotid bulb and internal carotid artery were measured (Table 2). The mean measurement was 0.83 and 0.80 mm for the right and left aforementioned vessels. There was no evidence of atheromatous plaque. Her endothelial function was assessed by a non-invasive technique, flow mediated dilatation, (FMD). The flow-mediated dilatation was 20% and the GTN-mediated dilatation was 16% post GTN, reflecting good endothelial function.

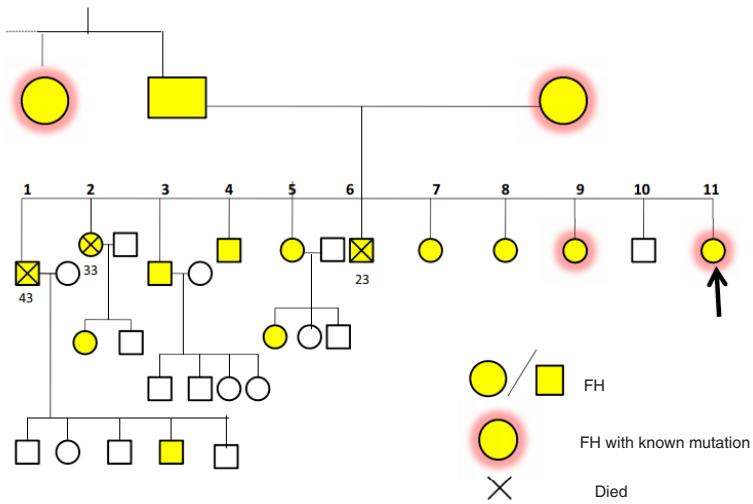


FIG. 2: Pedigree of patient's family showing most of family members are affected with FH. The arrow indicates the proband.

TABLE 1: Lipid screening (Total Cholesterol, TC and Low Density Lipoprotein Cholesterol (LDL-C) levels of patient's family members. 10 out of 16 screened family members have TC > 7.5 mmol/L and LDL-c > 4.9 mmol/L.

Relationship with patient	Age (yr)	TC (mmol/L)	LDL-C (mmol/L)	Lipid stigmata	Mutation/Genotype
First-degree relatives					
Father	63	8.1	5.7	Corneal arcus	No DNA sample
Mother	65	8.7	6.7	Corneal arcus; Xanthoma: *Achilles tendon*	Heterozygous c.763T>A
Sibling					
1	Died at 43	-	-	-	-
2	Died at 33	-	-	-	-
3	40	8.1	5.7	-	No mutation
4	39	8.0	6.4	-	No mutation
5	38	16.2	14.8	Xanthoma: *elbow*	No mutation
6	Died at 23	-	-	-	-
7	33	6.5	4.6	-	No mutation
8	32	7.0	5.2	Corneal arcus	No mutation
9	26	10.1	6.7	Corneal arcus, xanthoma: *Achilles tendon*	Heterozygous c.763T>A
11	23	4.9	2.6	-	No mutation
Second-degree relatives					
Paternal aunt	50	7.5	6.08	Corneal arcus; xanthoma*: Achilles tendon*	Heterozygous c.763T>A
Niece (daughter to sibling 1)	12	6.1	4.4	-	No DNA sample
Nephew (son to sibling 2	13	8.7	6.5	-	No DNA sample
Niece (daughter to sibling 5)	11	7.7	4.6	-	No DNA sample

TABLE 2: Carotid intima media thickness, (cIMT) of patient

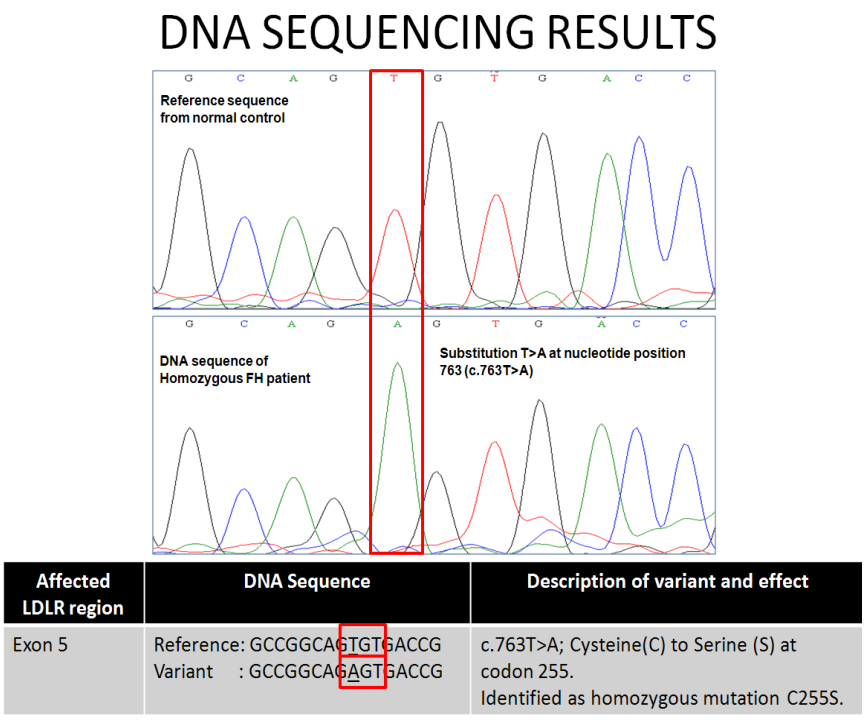
	Right Size (mm)	Left Size (mm)
Common carotid artery	0.82	0.78
Carotid bulb	0.85	0.82
Internal carotid artery	0.81	0.80
Mean	0.83	0.80

Her blood was sent for genetic study specifically looking at mutations in the LDLR gene and specific known mutation sites of Exon 26 and Exon 29 in the APOB gene. The DNA screening of LDLR and APOB genes was done by Polymerase Chain Reaction (PCR), followed by Denaturing High Performance Liquid Chromatography (DHPLC). She was found to have homozygous mutation C255S in Exon 5 affecting the ligand binding domain of the LDLR gene (Figure 3), with a change in the amino acid Cysteine to Serine. Using polymorphism phenotyping (Polyphen) software, the substitution is predicted to be 'probably damaging' and also identified by another protein prediction software SIFT, as 'not be tolerated'. Meanwhile, three other family members (mother, paternal aunt and her elder sister) were heterozygous for this mutation (Figure 4). No mutation of APOB gene (Exon 26 and 29) was found in the family. Throughout her follow-up at the clinic, she was put on several statins including pravastatin, simvastatin, atorvarstatin including an adjunct therapy, ezetimibe. She did not have major side effects of the prescribed drugs. All CK and ALT were monitored. She had also been counseled to have strict low cholesterol diet and advised for regular exercise. Along the course of the treatment, the lowest LDL-C achieved was 9.8 mmol/L with TC of 11.8 mmol/L.

DISCUSSION

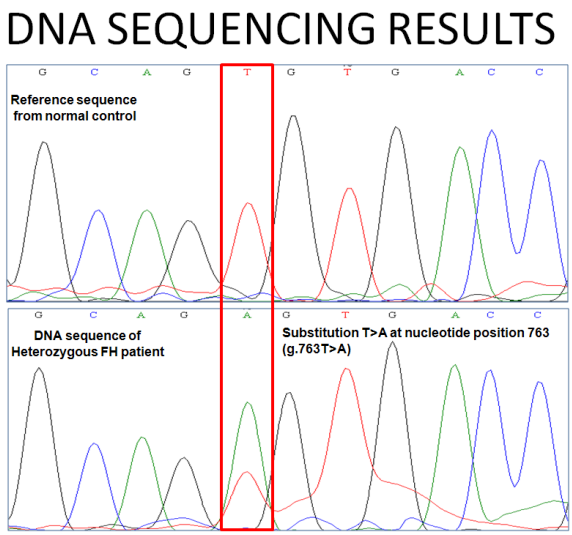
The mode of inheritance of FH was first described by Khachadurian in Lebanon and was defined as an autosomal dominant disease based on phenotypic severity of "heterozygous" and "homozygous" forms, with serum LDL-C levels which are two times and four times that of normal, respectively.[5] Patients with homozygous FH typically present as young children with markedly elevated cholesterol levels, very resistant to lipid lowering agents. In contrast, HeFH is usually diagnosed in adults and often respond well to medical therapy. In relation to this case, the patient was confirmed to have homozygous mutant alleles based on genetic analysis. However, she did not present at a young age as typically described in the literature.

There are several criteria used and established in the diagnosis of FH such as the MedPed criteria (USA),[6] the Simon Broome Register criteria (UK),[4] and the Dutch Lipid Clinic Network criteria (Netherlands).[7] To date, there is no individual diagnostic test with sufficient specificity and sensitivity to reliably detect FH.[8,9] The US MEDPED criteria are solely depending on LDL-C level and age. As compared to the US



Affected LDLR region	DNA Sequence	Description of variant and effect
Exon 5	Reference: GCCGGCAGTGTGACCG Variant : GCCGGCAGAGTGACCG	c.763T>A; Cysteine(C) to Serine (S) at codon 255. Identified as homozygous mutation C255S.

FIG. 3: DNA sequencing result of affected LDLR region at exon 5 showing homozygous mutation C255S.



Affected LDLR region	DNA Sequence	Description of variant and effect
Exon 5	Reference: GCCGGCAGTGTGACCG Variant : GCCGGCAGAGTGACCG	g.763T>A; Cysteine(C) to Serine (S) at codon 255. Identified as heterozygous mutation C255S.

FIG. 4: DNA sequencing result of affected LDLR region at exon 5 showing heterozygous mutation C255S

criteria, which used only lipid levels, the UK and Dutch criteria include family history, personal history, and physical signs and genetic testing in addition to blood cholesterol levels.[10] We used Simon Broome Register Group criteria to diagnose FH in this patient, as recommended by The National Institute for Health and Clinical Excellence (NICE) guidelines on the identification and management of familial hypercholesterolemia.[8] Based on her LDL-C level and the presence of tendon xanthoma, she was clinically diagnosed as definite FH. With the availability of genetic testing at our center, she was confirmed to have a c.763T>A mutation in which a substitution of T>A, led to an amino acid change of Cysteine (C) to Serine (S) in nucleotide position 763 in exon 5 of the LDLR gene.

Clinical features of FH are related to its metabolic peculiarities that persist from very early childhood, leading to accumulation of cholesterol in the form of xanthoma in skin and tendons and atheromatous lesions in the arterial wall, in particular, in the aorta and the stem of coronary arteries. Xanthelasma and premature corneal arcus are less useful, but their presence should prompt investigations.[8,9] In this patient, it was noted that most of the family members had xanthelasma but there was unawareness of the significance of the physical sign. Unfortunately, even though there was a history of premature sudden cardiac death in 3 members in the family, the cascade screening was not done, thus they remained undiagnosed. By employing B mode ultrasound, a non-invasive, widely available technology, the combined width of the carotid artery intima and media, can be readily visualized in almost all subjects. The normal carotid artery wall is unaffected by age or gender until approximately 18 years of age. However, the difference in mean cIMT between children with FH and their unaffected siblings may be significant as early as age 8 years.[11] It is common practice to regard carotid IMT >1.0mm as being abnormal. However, the normal values need to be adjusted for age, gender and perhaps even ethnicity. In a study done in UK in a healthy population, the upper limits (97.5 percentile) of IMT at CCA for participants age 35 to 39 years, 40 to 49 years, 50 to 59 years, and 60 years or older were 0.60, 0.64, 0.71, and 0.81 mm, respectively, whereas for that at the bifurcation were 0.83, 0.77, 0.85, and 1.05 mm, respectively.[12] In regards to this patient, with mean cIMT of 0.83 and 0.8mm on the right and left carotid artery, respectively, it is concluded that there is an increased thickness of cIMT in respect to her age which suggests an early risk to develop atherosclerosis. The atheromatous plaque is not visible in this case through this ultrasound technique probably because the effect

of hypercholesterolemia on plaque formation takes longer time to develop than the effect of hypercholesterolemia on intima media thickness. Her endothelial function was also assessed. It is known that arterial endothelial dysfunction is one of the early events in the atherogenesis, preceding structural atherosclerotic change. It can be accessed through a non-invasive technique, using High-resolution B-mode ultrasound. It provides valuable insight into early atherogenesis, as well as into the potential reversibility of endothelial dysfunction, including pharmacological agents (lipid lowering, ACE inhibition), L-arginine, antioxidants and hormones. In this technique, brachial artery diameter is measured during 3 conditions; baseline, during reactive hyperemia and after administration of sublingual nitroglycerin, (GTN). The flow-mediated dilation is expressed as a percentage change of diameter after reactive hyperemia relative to the baseline scan. Likewise, the GTN-mediated dilation is expressed as a percentage change of diameter after GTN administration relative to the baseline scan.[13]

Mutation in four genes has been identified to have implication on the low density lipoprotein receptor (LDLR) pathway: (a) LDLR gene (b) B-100 (APOB-100) gene that encodes for the apolipoprotein component of LDL particles, (c) proproteinconvertasesubtilisin/kexin type 9 (PCSK9) gene and (d) autosomal recessive hypercholesterolemia (ARH) gene.[14] The presence of other candidate genes has been postulated, but these are rare.[15] In regards to this patient, she was found to have a mutation in exon 5 affecting the ligand binding domain of LDLR gene. This mutation has also been previously described by Azian *et al* in the Malaysian population.[16] APOB mutation cannot be clinically distinguished from HeFH;[17] therefore, a genetic testing is required so that suitable treatment and cascade screening can be performed for the patient's family members.[18] Management for FH involves diet therapy, lifestyle modification and pharmacotherapy. To date, statins are by far the most common and effective drugs to treat FH.[19,20] Higher-risk FH patients that require greater lowering of plasma LDL-C and apoB may benefit from another group of lipid lowering drugs, especially ezetimibe, niacin, fenofibrate and bile-acid binding resins.[21,22] Several guidelines recommend the target LDL-C for FH patients. Based on guidelines from the US National Lipid Association (NLA) and NICE in the UK, a reduction in LDL-C concentration of 50% from levels before treatment is recommend in patients with FH.[8] This has not yet been achieved in this patient.

LDL-C apheresis is an extracorporeal removal of apoB-containing lipoproteins from the circulation. It is usually indicated for patients with homozygous or compound heterozygous FH, as well as for patients with heterozygous FH with documented CHD who are refractory to pharmacotherapy.[19] It is typically run at fortnightly intervals which can acutely reduce LDL-c to 75% reduction with minimal side effects reported.[23] However, in Malaysia, the availability of lipid apheresis is very limited and it is very costly amounting around RM 3000 per session. Nowadays, several new classes of pharmacotherapy have been developed to lower down LDL-C into target level. These include inhibitors of PCSK9, microsomal triglyceride transfer protein (MTTP), cholesteryl ester transfer protein (CETP) and mipomersan; an antisense apoB synthesis inhibitor. Recently, the Food and Drug Administration (FDA) approved two new treatments as adjunct therapy for the indication of HoFH. It is good to know that there are novel emerging therapies for treating this serious disease. It is hoped that these drugs would be available soon in Malaysia and more HoFH patients would benefit from the treatment.

Underdiagnosed FH is a global challenge. It is not known why patients with FH are often missed in primary care, but many seemed to be diagnosed in middle age when family members present with coronary heart disease. Although patients diagnosed with FH are instructed to contact their relatives, several studies have shown that this is not effective in practice.[24] In Malaysia, the lack of national screening programs, inadequate recording of family history and minimal resources for genetic testing probably contribute to this matter. Campaigns to increase awareness of this disease should be highlighted and more educational programs should be implemented not only to the public but also to the health care personnel.

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The patient has no objection to publication of this article for educational purposes.

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