A Novel Mutation in Exon 5 of the Low Density Lipoprotein Receptor Gene in a Malay Family with Familial Hypercholesterolaemia (FH)

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ABSTRACT

Familial hypercholesterolaemia (FH) is an autosomal dominant inherited disease of lipid metabolism caused by mutations in the *low density lipoprotein receptor (LDLR)* gene. FH is clinically characterised by an elevated concentration of total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C), the presence of xanthomata and premature atherosclerosis. The objective of this study was to characterise the *LDLR* gene mutations in members of a family with FH. In total, 24 individuals were enrolled into this study. A proband from this family was diagnosed as FH based on the Simon Broome's criteria. Mutational screening was performed by polymerase chain reaction - denaturing gradient gel electrophoresis (PCR-DGGE) approach. Those bands that shifted on DGGE were subjected to DNA sequencing to confirm the mutation. We identified a base substitution, T to A at position 763 resulting in substitution of amino acid cysteine (C) to serine (S) at codon 234. This mutation was detected in exon 5 of the *LDLR* gene which involved the ligand binding domain and is designated as C234S mutation. This domain is important for the binding of LDLR to its ligand, apolipoprotein B100, in order to regulate the LDL catabolism through the LDLR mediated pathway. Mutation in this region may reduce the binding affinity of the LDLR to apolipoprotein B100. To our knowledge, this is a novel mutation worldwide. This mutation could possibly have important clinical implications in view of the high incidence of coronary artery disease (CAD) and sudden cardiac death (SCD) in the family.

INTRODUCTION

The low density lipoprotein (LDL) particles are catabolised through the low density lipoprotein receptor (LDLR) pathway and is mediated by the interaction with apolipoprotein B-100 (1, 2). Mutations in the LDLR and its ligand, apo B-100 will diminish the LDL catabolism through the LDLR mediated pathway resulting in Familial Hypercholesterolaemia (FH) or Familial Defective Apolipoprotein B-100 (FDB) (3). Both are autosomal dominant inherited diseases with a clear gene dosage effect (3, 5). FH is clinically characterized by an elevated serum concentration of total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C), the presence of xanthomata and premature atherosclerosis (6, 7). Patients heterozygous and homozygous for the *LDLR gene* mutation show a great variability in their phenotypic expression. This may be due to the diversity of mutations in the *LDLR* gene which results in non-functional or partially functional LDLR (6, 7). FH homozygotes inherit two abnormal *LDLR* gene with no LDLR function leading to a four to five fold increase in the LDL-C and atherosclerosis commences during childhood (6, 7). Heterozygotes inherit one normal and one defective *LDLR* gene and have two to three fold elevated plasma LDL-C levels and generally develop late onset atherosclerosis (6, 7). On the other hand, mutations in the *apo B-100* gene also may result in elevated LDL-C concentrations and the phenotype can be clinically indistinguishable from FH (8, 9). This condition is designated as Familial Defective Apolipoprotein B-100 (FDB). The biochemical and clinical characteristics of FDB includes moderate to severely increased LDL-C, presence of tendon xanthoma, corneal arcus and premature coronary artery disease (CAD). The prevalence of FH and FDB is estimated to be 1 in 500 to 700 in most populations worldwide (10). About 2-5% of FH patients may actually have FDB (9). More than 700 mutations in the *LDLR* gene and 3 common mutations in the *apo B-100* gene have been identified worldwide (5, 11). The spectrum of mutations varies in certain populations due to the founder effect,

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such as those mutations found among the Finns in Finland, Jews in Israel, Afrikaners in South Africa, Lebanese Christians and French Canadians (12-16). However, the *LDLR* gene mutations in our heterogenous multi-ethnic Malaysian population have not been fully characterized. Traditionally, FH is biochemically diagnosed by elevated serum LDL concentration in the presence of tendon xanthomata (12). However, about 15% - 20% of the affected relatives may be missed by cholesterol testing alone (4). Hence, the DNA analysis is important for the definitive diagnosis of FH and to characterise the mutations particularly in the setting of family studies. This may lead to early detection in family members thus could prevent mortality due to hypercholesterolaemia and their complications such as cardiovascular diseases. Therefore, the aim of this study was to identify and characterize the *LDLR* and/or *apo B-100* genes mutation among the members of a Malay family with FH using the Polymerase Chain Reaction – Denaturing Gradient Gel Electrophoresis (PCR-DGGE) approach.

MATERIALS AND METHODS

STUDY POPULATION

A proband from this family was identified at the Lipid Clinic, Universiti Kebangsaan Malaysia Hospital (HUKM). This patient was classified as definite FH based on the Simon Broome's criteria (7). Definite FH is defined as serum total cholesterol (TC) levels of $\geq$ 7.5 mmol/L and/or LDL cholesterol of $\geq$ 4.9 mmol/L (age > 16 years), TC > 6.5 mmol/L (age $\leq$ 16 years) and tendon xanthomata either in at least 1 family member i.e. the patient him/herself and/or his/her first or second degree relatives. Non-FH among the family members is defined as TC $\geq$ 5.2 mmol/L but less than 7.5 mmol/L with negative mutation screening while normal is defined as those with TC < 5.2 mmol/L. Whole blood samples were collected in EDTA-anticoagulated tubes and fasting sera were collected in plain tubes after overnight fasting. Blood samples from normocholesterolaemic controls (TC< 5.2 mmol/L) were also collected in parallel. Samples of known *LDLR* and *apo B-100* gene mutations (positive controls) were obtained from other FH researchers. Ethics approval for the study was obtained from the UKM Research and Ethics Committees. In total 24 individuals from this family took part in this study and gave written informed consent for their participation.

BIOCHEMICAL ANALYSIS

All 24 family members underwent a screening process which consisted of clinical history, physical examination and laboratory tests including fasting glucose (FG), fasting serum lipid (FSL), renal profile (RP), liver function test (LFT) and thyroid function test (TFT). Serum samples were analyzed for TC, HDL-C and TG using the standard laboratory procedures. The serum samples were also assayed for FG, RP, LFT and TFT by routine laboratory methods. Fasting blood samples were analyzed for glucose level using the hexokinase enzymatic reference method on the Cobas Integra 700 (Roche Diagnostics, Basel, Switzerland). TC, TG and HDL-C were measured by enzymatic reference methods also on the Cobas Integra 700. LDL-C concentration was determined using the Friedweld equation, LDL-C = TC - (HDL-C + TG/2.2) [18].

DNA EXTRACTION

Total genomic DNA was isolated using a commercial kit (Roche Diagnostics, Basel, Switzerland) using the method as described by the manufacturer. The isolated DNA samples were electrophoresed on 1.2% agarose gels and the purity was determined using a spectrophotometer (A260/A280).

POLYMERASE CHAIN REACTION (PCR) – DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE)

The 3456-3553 region of the *apo B-100* and all 18 exons of the *LDLR* gene including the promoter region were screened in all 24 subjects. PCR was performed using oligonucleotide primers as previously described by Nissen *et al*. (1996) (11) as shown in Table 1. PCR mixtures were prepared in a total volume of 50 µl consisting of: 100-500 ng DNA, 1.5-3.0 mM $MgCl_2$, 0.2 mM dNTPs, 10x PCR Buffer, 10-20 pmol oligonucleotide primers and 2.5 U Taq polymerase (Platinum Taq, Invitrogen). PCR was carried out using iCycler Thermal

Table 1. Oligonucleotide primers for amplification of the promoter region and the 18 exons of the human *LDL receptor* gene and the codon 3500 region of the human *Apolipoprotein B-100* gene

	LDLR	Forward Primer (5')	Reverse Primer (3')	Base pair	DGGE
1.	Promoter	[b]AGGACTGGAGTGGGAATCAGAGC	TGCTGTGTCCTAGCTGGAAACCC	252	-
2.	Exon 1	[a]TTGAAATGCTGTAAATGACGTGG	CTGGCGCCTGGAGCAAGC	256	30-70
3.	Exon 2	[b]CGTGGTCAGTTTCTGATTCTGGCG	ATAAATGCATATCATGCCCAAAGG	253	30-70
4.	Exon 3	[b]TCGGCCTCAGTGGGTCTTTC	ACTCCCCAGGACTCAGATAGGC	268	30-70
5.	Exon 4-5'	[b]ACTGCGGCAGCGTCCCCGGC	GGATGCAGGTGGAGCTGTTGC	297	20-80
6.	Exon 4-3'	ACCTGTGGTCCCGCCAGC	[b] CCAGGGACAGGTGATAGGACG	345	-
7.	Exon 5	[b]GGCCCTGCTTGTTTTTCTCTGG	AGCAGCAAGGCACAGAGAATGG	282	20-80

continue

Table 1 (Continued)

LDLR	Forward Primer (5')	Reverse Primer (3')	Base pair	DGGE
8. Exon 6	[b]ACGAAACTGAGGCTCAGACACACC	GCTCCCCACAAACTCTGCAAGC	262	20-80
9. Exon 7	[b]AGAGTGACCAGTCTGCATCCCTGG	TTGGTTGCCATGTCAGGAAGC	253	35-65
10. Exon 8	[b]TCCCCACCAAGCCTCTTTCTCTC	CCACCCGCCGCCTTCC	222	30-70
11. Exon 9	[c]CTGACCTCGCTCCCCGGACC	GGCTGCAGGCAGGGGCGACG	278	30-70
12. Exon 10	GCAGTGAGATGAGGGCTCCTGG	[b] CCTGCAGCCCTCAGCGTCG	349	30-70
13. Exon 11	[b]GGATCCTCCCCCGCCCTC	TGGCTGGGACGGCTGTCC	239	30-70
14. Exon 12	GGCCCTCAGGCCCTCTGG	[b]CCGAGTTTTCTGCGTTCATCTT	336	30-70
15. Exon 13	[a]GTCATCTTCCTTGCTGCCTG	CACAAGGAGGTTTCAAGGTTGG	264	20-60
16. Exon 14	[a]TCTCGTTCCTGCCCTGACTCC	GACACAGGACGCAGAAACAAGG	274	30-70
17. Exon 15	[b]GGCACGTGGCACTCAGAAGACG	[a]GTGTGGTGGCGGGCCCAGTC	288	30-70
18. Exon 16	[a]CTCCATTTCTTGGTGGCCTCCC	CATAGCGGGAGGCTGTGACCTGG	239	30-70
19. Exon 17	[a]GGGCAGCTGTGTGACAGAGCG	CATGGCTCTGGCTTTCTAGAGAGG	279	30-70
20. Exon 18	[a]CCTGAGTGCTGGACTGATAGTTTCC	AAGGCCGGCGAGGTCTCAGG	190	-
21. Apo B100	[b]GGAGCAGTTGACCACAAGCTTAGC	GGTGGCTTTGCTTGTATGTTCTCC	382	30-70

[a]50-bp GC-clamp: CGCCCGCCGCCGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG
[b]40-bp GC-clamp: CGCCCGCCGCGCCCCGCGCCCGTCCCGGCCGCCCCCCCGCCCG
[c]46bpGC/10bpAT:CGCCCGCGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCGAAATAATAA
[d]3-bp GC-clamp: CGG
[e]Apolipoprotein B codon 3456-3553 (Nissen *et al.,* 1995c)

Cycler (Bio-Rad, USA). DNA template was initially denatured for 5 mins at 94°C, followed by 35 PCR cycles as follows: denaturation for 15 to 30s at 95°C, annealing for 13 to 45s at 55°C to 70°C and elongation for 13 to 60s at 72C followed by extension at 72°C for 5 to 10 mins. The cycle was repeated 35 times and the PCR conditions were optimised according to the length of the amplicon to be amplified. DGGE was performed to identify single base changes in a segment of DNA. PCR products were heated at 95°C for 5 minutes, placed at 65°C for 1 hour and then slowly cooled at room temperature to generate the heteroduplex or homoduplexes molecules. The heteroduplexes or homoduplexes were electrophoresed using a 6% gel and 20-80% denaturing solution (depending on each exon), at 130V, 60°C for 4 to 6 hours (depending on each exon). Finally, the gels were stained with ethidium bromide and visualised using a digital camera system (Kodak, USA).

CONFIRMATION OF MUTATION BY DNA SEQUENCING

Samples with band shifts were subjected to DNA sequencing for confirmation of the mutations. PCR was repeated using oligonucleotide primers without GC clamps. PCR products were purified using QIAquick Gel Extraction kit (Qiagen, Germany) and subsequently electrophoresed on a 1.2% agarose gel at 100V for 1 hour. The cycle sequencing was performed at 96°C for 10 minutes, 50°C for 5s and 60°C for 10s for 25 cycles. The cycle sequencing products were purified using ethanol/sodium acetate precipitation method. The DNA was suspended in formamide and DNA sequencing was performed by ABI PRISM 3100 Genetic Analyzer. The results were analyzed using Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov). DNA sequencing was directly performed for the promoter region, exon 4-3' and exon 18 due to difficulties in optimising the DGGE technique.

RESULTS

FAMILY HISTORY OF HYPERCHOLESTEROLAEMIA, SUDDEN CARDIAC DEATH AND PHYSICAL FEATURES OF FH

The proband from this family was diagnosed as a definite FH patient in the Lipid Clinic, HUKM. She came from a consanguineous marriage where her father and mother were first cousins. There was a strong family history suggestive of hypercholesterolaemia and sudden cardiac deaths in this family. She has 11 siblings but 3 of them died of sudden cardiac death due to premature cardiovascular diseases. Her first brother died at aged 43 years due to stroke and had high levels of cholesterol. Her first sister also died at a young age (32 years) due to cardiovascular disease. In addition, her fourth brother died at age 23 years due to myocardial infarction (MI). Physical features of FH including xanthelasma, corneal arcus and xanthomata (Figure 1) were observed in the family members. The proband had xanthelasma around her eyelids as shown in Figure 1. Four of the family members had xanthomata (3 over the Achilles tendon, 3 on the dorsum of the hand). Seven of the family members had corneal arcus (3: grade 1, 1: grade 2, 1: grade 3, 2: grade 4).

BIOCHEMICAL ANALYSIS (FASTING LIPID PROFILE)

In total, 24 members from this family were screened for mutations in the *LDLR* gene. These include the first and

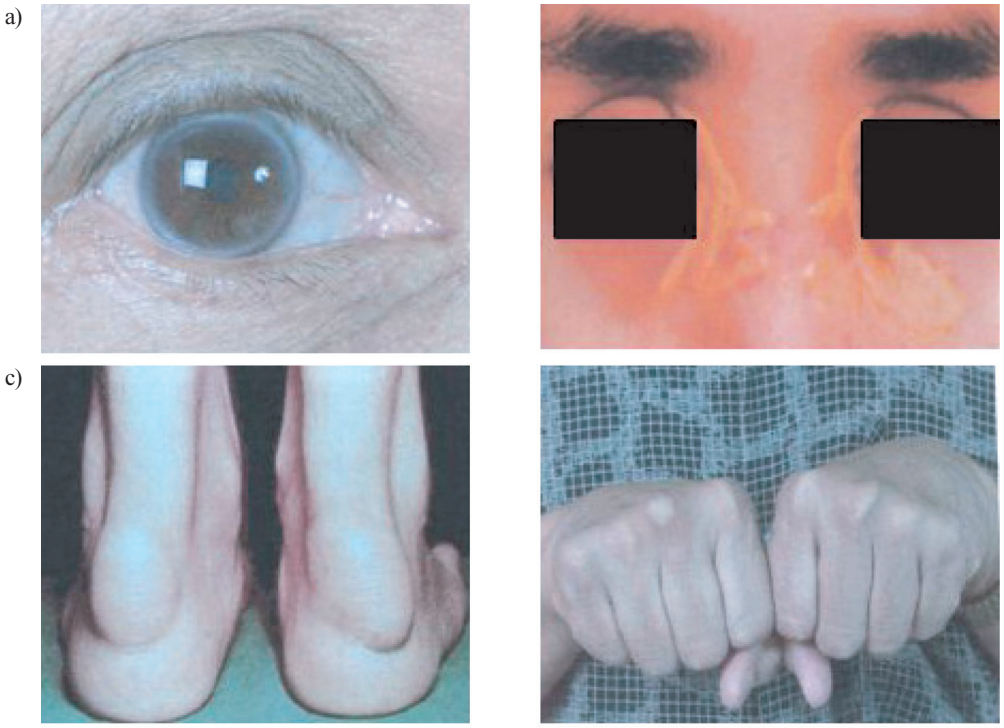

Figure 1. Clinical features of FH patients. a) Corneal arcus grade 4, a greyish-white ring (or part of a ring) opacity occurring in the periphery of the cornea which is due to a lipid infiltration of the corneal stroma. b) Xanthelasma, a cutaneous deposition of lipid material that appears in the skin of the eyelids, as a yellowish and slightly elevated area. c and d) Tendon xanthomata on the knees and fingers which is an elevated or rounded, yellowish or orangish nodules on the skin over joints, especially on elbows and knees.

second degree relatives of the proband (FH 001) as shown in the family pedigree (Figure 2a and 2b). Based on the biochemical and clinical criteria, 10 patients were identified as definite FH, 5 were non-FH hypercholesterolaemia and 9 were normocholesterolaemia individuals. The biochemical and clinical characteristics of these patients are shown in Table 2. The total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) for the definite FH (n = 10) were 9.7 $\pm$ 3.3, 1.3 $\pm$ 0.8, 7.9 $\pm$ 3.6 and 1.2 $\pm$ 0.3 mmol/L, respectively. TC, TG, LDL and HDL levels for non FH (n = 5) were 6.3 $\pm$ 0.3, 0.8 $\pm$ 0.1, 4.5 $\pm$ 0.3 and 1.4 $\pm$ 0.1 mmol/L, respectively. For the unaffected family members (n = 9) were 4.2 $\pm$ 0.9, 1.0 $\pm$ 0.5, 2.4 $\pm$ 0.8 and 1.1 $\pm$ 0.3 mmol/L respectively.

LDLR GENE MUTATION

We successfully amplified all exons of the *LDLR* gene using the PCR method. Figure 3a) represents the PCR analysis of exon 5 and shows a band at position 282bp. Mutational analysis by PCR-DGGE on exon 5 showed a band shift suggesting the presence of a mutation in the samples as indicated in Figure 3b (lane 7 and 8). DNA sequencing revealed a substitution of nucleotide T to A at 763 resulting in substitution of amino acid cysteine (C) to serine (S) at codon 234. This mutation is designated as C234S. We identified 11 heterozygotes and 1 homozygote from the DNA sequencing analysis as shown in Figure 4. This mutation was possibly inherited from both parents of the proband. Six of the proband's siblings were heterozygous of the mutation. One sister was homozygous and showed physical and biochemical features of FH such as corneal arcus, grade 3, xanthomata at the Achilles tendon and the dorsum of the hand as well as TC = 16.2 mmol/L and LDL = 14.8 mmol/L. She was diagnosed with peripheral vascular disease (PVD) during this study. Her eldest son was also confirmed as a heterozygote individual with TC = 7.7 mmol/L and LDL = 6.0 mmol/L. No mutation was observed in the *apo B-100* gene in all family members.

DISCUSSION

This study was performed to characterize the *LDLR* gene mutation in a family with FH. The proband had features of definite FH when she was referred to the Lipid Clinic, HUKM. Family screening was performed in order to detect the mutation among the first and second degree relatives. The purpose was to initiate early treatment in those members with FH in order to reduce mortality and morbidity due to premature atherosclerosis-related diseases. Family screening was only performed on the paternal side since there was no other sibling from the maternal side and both maternal parents had deceased.

We identified a C234S mutation in the exon 5 of the *LDLR* gene in this family. This mutation is a missense mutation at nucleotide 763. The T to A substitution at nucleotide 763 and the codon region 234 results in

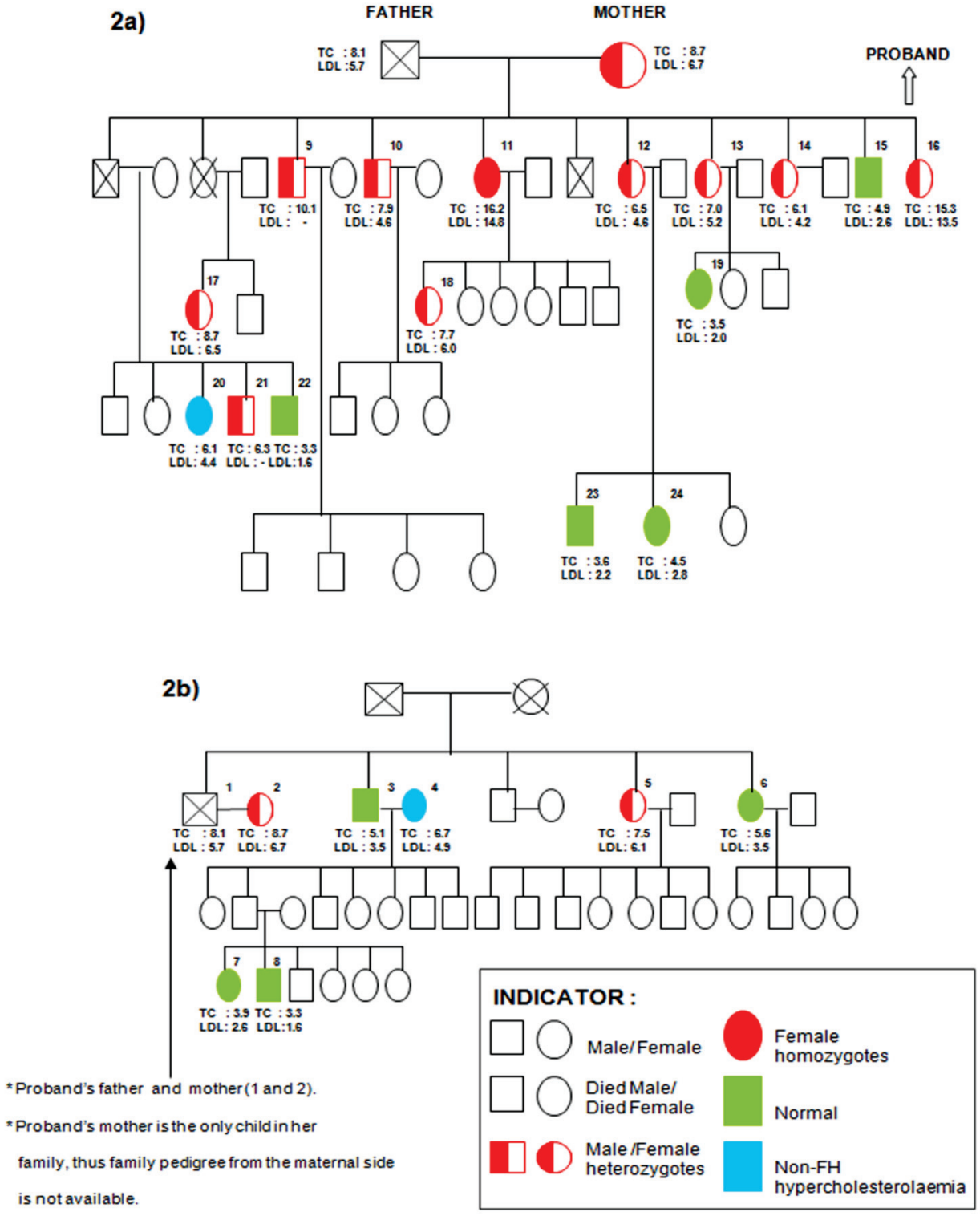

Figure 2. Family pedigree of the patient diagnosed with FH. a) The proband from this family is a female, 35 years old. Family tree shows her first degree relatives. b) Family tree from a paternal side (father) of the patient revealed her second degree relatives. Altogether, 24 individuals were involved in this study.

Table 2. Lipid profiles of members of this family according to definite FH, NFH and normal control groups based on the Simon Broome's criteria

	Definite FH	NFH	NC
[#] n/ (F/M)	10/ F = 7, M = 3	5/ F = 4, M =1	9/ F = 2, M = 7
*Age (years)	41.3 ± 17.3	28.4 ± 10.5	20.8 ± 19.5
*TC (mmol/L)	9.7 ± 3.3	6.3 ± 0.3	4.2 ± 0.9
*TG (mmol/L)	1.3 ± 0.8	0.8 ± 0.1	1.0 ± 0.6
*LDL (mmol/L)	7.9 ± 3.6	4.5 ± 0.3	2.4 ± 0.8
*HDL (mmol/L)	1.2 ± 0.3	1.4 ± 0.1	1.2 ± 0.3

Data are expressed as proportion ([#]) and mean ± SD (*)

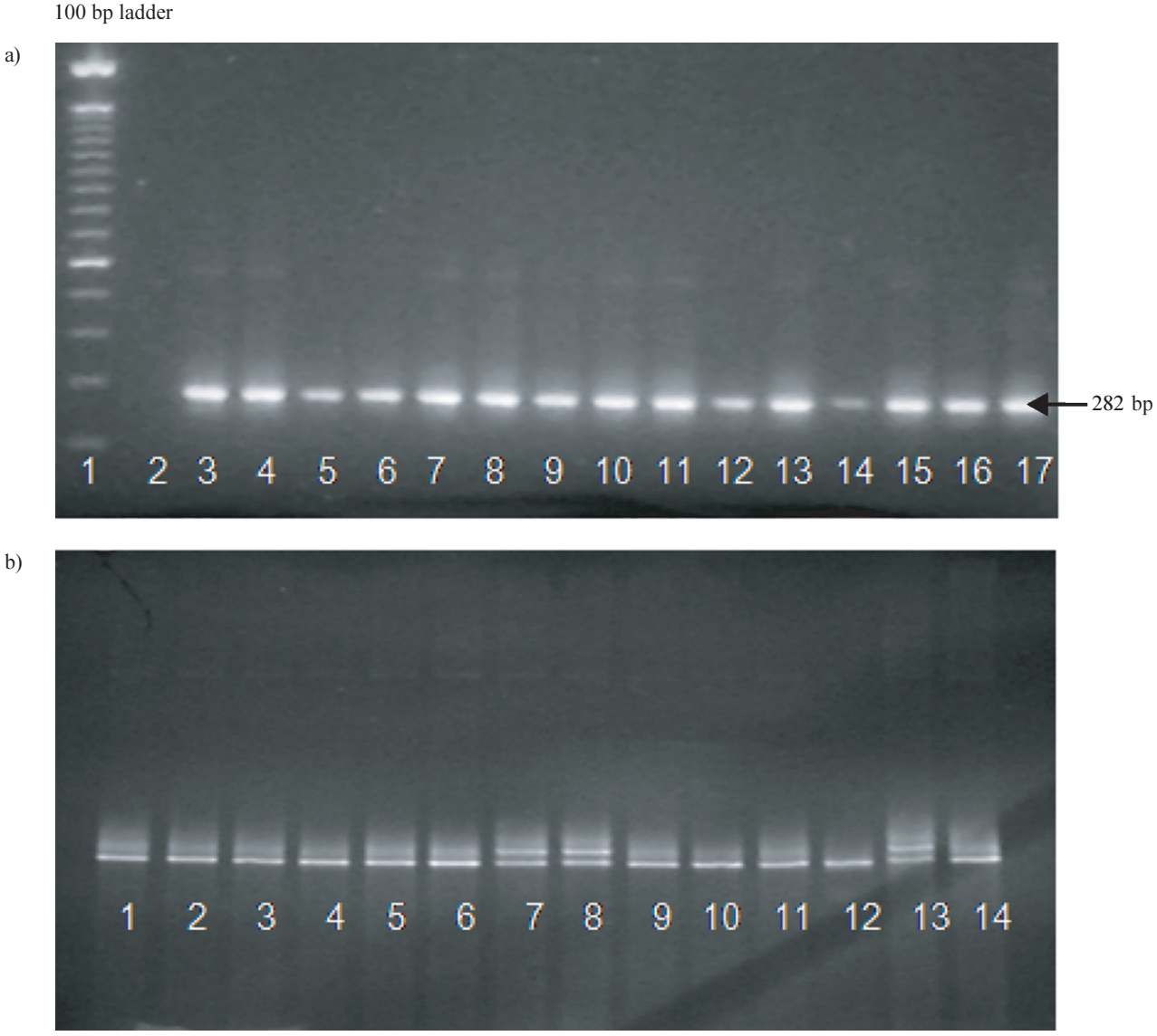

Figure 3. DGGE analysis. a) PCR of exon 5 of the *LDLR* gene showed a band at 282bp. b) Possible mutations in exon 5 of the *LDLR* gene. Lane 14 was normal control, lane 13 (positive D245E mutation), lane 7 and 8 showed band shifts suggesting mutations were present in these samples.

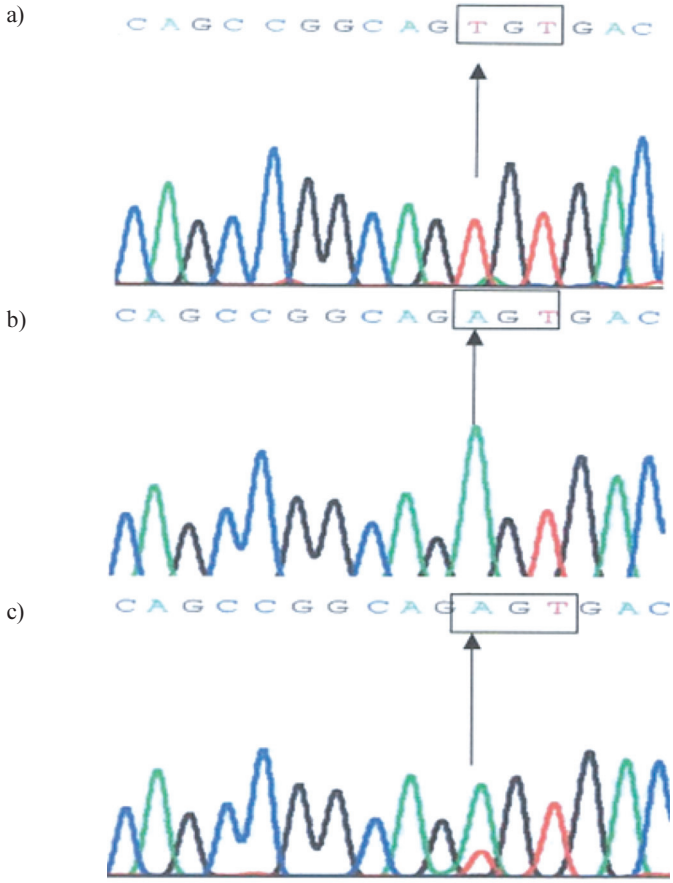

Figure 4. Electropherogram of the DNA sequencing results of 5 of the *LDL receptor* gene. a) Top and bottom panels were normal and mutated sequences respectively. b) A substitution of TGT > AGT in exon 5 resulting in C234S homozygous mutation. c) Heterozygous C234S mutation showed by a mixture of A/T nucleotides.

Table 3. Clinical and biochemical characteristics of the heterozygous and homozygous C234S mutation of exon 5 and the unaffected family members

		Heterozygous mutation	Homozygous mutation	Unaffected family members	P value(Htz vs unaffected family members)
1.	#n (F/M)	11 / F = 7, M = 4	1 / F = 1	9/F = 3, M = 6	-
2.	*Age (years)	38.91 ± 17.93	40	20.8 ± 19.5	0.052
3.	*TC (mmol/L)	8.52 ± 2.61	16.2	4.2 ± 0.9	0.000
4.	*TG (mmol/L)	1.35 ± 0.80	0.6	1.0 ± 0.5	0.825
5.	*LDL (mmol/L)	6.74 ± 0.88	14.8	2.4 ± 0.8	0.000
6.	*HDL (mmol/L)	1.25 ± 0.28	1.1	1.1 ± 0.3	0.307
7.	Xanthomata	4/11 (36.4%)	Present	Absent	-
8.	Xanthelasma	2/11 (18.1%)	Absent	Absent	-
9.	Corneal arcus	8/11 (72.7%) (grade 1: 3 individuals, grade 2: 1 individual, grade 4: 2 individuals)	Present (grade: 3)	Absent	-
10.	CAD	1/11 (9%)	Absent	Absent	-
11.	PVD	Absent	Present	Absent	-
12.	Stroke	Absent	Absent	Absent	-

Data are expressed as proportion (#) and mean ± SD (*)

substitution of amino acid cysteine (C) to serine (S). To our knowledge this mutation is a novel mutation which has not been reported by other studies. Based on the gene structure, this mutation affects the ligand binding domain of the *LDLR* gene. It is important for the LDL receptor to bind to its ligand which is the Apolipoprotein B100 which subsequently will enhance the LDL uptake and catabolism through the LDL receptor-mediated pathway. Mutation in this region may reduce binding affinity of the LDLR to the Apo B-100. However, no *apo B-100* gene mutation was identified in this family.

In this study, a family member who was first diagnosed as non-FH hypercholesterolaemic patient based on clinical and biochemical findings was later shown to be heterozygous FH by mutational analysis. We also identified this novel mutation in 2 young family members (age 12 and 18 years) with untreated total cholesterol levels of 6.5 mmol/L and 7.7 mmol/L respectively. Thus, the DNA analysis has an important role in providing a definitive diagnosis of FH, particularly in family studies. Definitive diagnosis allows initiation of early preventive and therapeutic measures to reduce risk of premature CAD. In addition, genetic counseling can also be offered to these affected individuals.

From the high incidence of premature SCD and CAD among the members of this family, we postulate that this novel mutation has important clinical implications. In addition to molecular testing, endothelial function assessment was performed to determine their risk to develop cardiovascular diseases. The results showed that the flow mediated dilatation (FMD) ranged from 0% - 12.79%. Four individuals had low FMD values compared to the normal (> 10%) suggesting that they are at high risk to develop the cardiovascular artery disease (CAD). The estimated 10 years CHD risk for patients with C234S mutation using Framingham Risk Score in this family ranged from 7% to 27%. They are at mild to moderate risk level of developing CHD.

Although the DGGE technique is laborious and time consuming, it is a reliable screening tool to detect mutation in a particular gene (17-19). Given the large allelic and genetic heterogeneity in FH, the mutation detection method requires a high-throughput, rapid, and affordable technique to integrate molecular screening into clinical practice efficiently. Given the advancement in sequencing technology nowadays, mutational analysis is much more convenient to perform and faster compared to the conventional gel-based method. For example, Hollants et al combined a microfluidic amplification system with massive parallel sequencing for mutation scanning in FH patients, which can be implemented for diagnostic purposes (20). In addition, Chiou and colleagues developed an array-based resequencing assay in three most common genes for FH such as *LDLR*, *apo B-100* and *proprotein convertase subtilisin/kexin type 9* genes (*PCSK9*) to facilitate genetic testing in FH patients (21).

In conclusion, we have identified a novel mutation in exon 5 (C234S) of the *LDLR* gene in a Malay family with FH and 12 of the 24 family members have the mutation. This mutation affects the ligand binding domain of the *LDLR* gene which possibly reduced the binding affinity of the LDL to the LDL receptor. Given the high occurrence of hypercholesterolaemia, history of cardiovascular diseases and sudden cardiac death in the family, this mutation have important clinical implications. DNA mutational analysis is an important adjunct to clinical and biochemical data in order to determine a definitive diagnosis of FH among family members for initiating early preventive and therapeutic measures to reduce risk of premature CAD.

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REFERENCES

1. Goldberg AC, Hopkins PN, Toth PP, Ballantyne CM, Rader DJ, Robinson JG, 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 Jun; 5(3 Suppl): S1-8.
2. Fahed AC, Nemer GM. Familial hypercholesterolemia: the lipids or the genes? Nutr Metab (Lond). 2011; 8(1): 23.
3. Hopkins PN, Toth PP, Ballantyne CM, Rader DJ. Familial hypercholesterolemias: prevalence, genetics, diagnosis and screening recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. J Clin Lipidol. 2011 Jun; 5(3 Suppl): S9-17.
4. Humphries SE, Galton D, Nicholls P. Genetic testing for familial hypercholesterolaemia: practical and ethical issues. QJM. 1997 Mar; 90(3): 169-81.
5. Lombardi MP, Redeker EJ, Defesche JC, Kamerling SW, Trip MD, Mannens MM, et al. Molecular genetic testing for familial hypercholesterolemia: spectrum of LDL receptor gene mutations in The Netherlands. Clin Genet. 2000 Feb; 57(2): 116-24.
6. Nissen H, Guldberg P, Hansen AB, Petersen NE, Horder M. Clinically applicable mutation screening in familial hypercholesterolemia. Hum Mutat. 1996; 8(2): 168-77.
7. Ose L. An update on familial hypercholesterolaemia. Ann Med. 1999 Apr; 31 Suppl 1: 13-8.
8. Tybjaerg-Hansen A, Steffensen R, Meinertz H, Schnohr P, Nordestgaard BG. Association of mutations in the

apolipoprotein B gene with hypercholesterolemia and the risk of ischemic heart disease. N Engl J Med. 1998 May 28; 338(22): 1577-84.
9. Myant NB. Familial defective apolipoprotein B-100: a review, including some comparisons with familial hypercholesterolaemia. Atherosclerosis. 1993 Dec; 104(1-2): 1-18.
10. Graham CA, McClean E, Ward AJ, Beattie ED, Martin S, O'Kane M, et al. Mutation screening and genotype:phenotype correlation in familial hypercholesterolaemia. Atherosclerosis. 1999 Dec; 147(2): 309-16.
11. Nissen H, Hansen AB, Guldberg P, Hansen TS, Petersen NE, Horder M. Evaluation of a clinically applicable mutation screening technique for genetic diagnosis of familial hypercholesterolemia and familial defective apolipoprotein B. Clin Genet. 1998 Jun; 53(6): 433-9.
12. Koivisto UM, Turtola H, Aalto-Setala K, Top B, Frants RR, Kovanen PT, et al. The familial hypercholesterolemia (FH)-North Karelia mutation of the low density lipoprotein receptor gene deletes seven nucleotides of exon 6 and is a common cause of FH in Finland. J Clin Invest. 1992 Jul; 90(1): 219-28.
13. Leitersdorf E, Tobin EJ, Davignon J, Hobbs HH. Common low-density lipoprotein receptor mutations in the French Canadian population. J Clin Invest. 1990 Apr; 85(4): 1014-23.
14. Leitersdorf E, Van der Westhuyzen DR, Coetzee GA, Hobbs HH. Two common low density lipoprotein receptor gene mutations cause familial hypercholesterolemia in Afrikaners. J Clin Invest. 1989 Sep; 84(3): 954-61.
15. Lehrman MA, Schneider WJ, Brown MS, Davis CG, Elhammer A, Russell DW, et al. The Lebanese allele at the low density lipoprotein receptor locus. Nonsense mutation produces truncated receptor that is retained in endoplasmic reticulum. J Biol Chem. 1987 Jan 5; 262(1): 401-10.
16. Reshef A, Nissen H, Triger L, Hensen TS, Eliav O, Schurr D, et al. Molecular genetics of familial hypercholesterolemia in Israel. Hum Genet. 1996 Nov; 98(5): 581-6.
17. Top B, Uitterlinden AG, van der Zee A, Kastelein JJ, Leuven JA, Havekes LM, et al. Absence of mutations in the promoter region of the low density lipoprotein receptor gene in a large number of familial hypercholesterolaemia patients as revealed by denaturing gradient gel electrophoresis. Hum Genet. 1992 Jul; 89(5): 561-5.
18. Lombardi P, Sijbrands EJ, van de Giessen K, Smelt AH, Kastelein JJ, Frants RR, et al. Mutations in the low density lipoprotein receptor gene of familial hypercholesterolemic patients detected by denaturing gradient gel electrophoresis and direct sequencing. J Lipid Res. 1995 Apr; 36(4): 860-7.
19. Azian M, Hapizah MN, Khalid BA, Khalid Y, Rosli A, Jamal R. Use of the denaturing gradient gel electrophoresis (DGGE) method for mutational screening of patients with familial hypercholesterolaemia (FH) and Familial defective apolipoprotein B100 (FDB). Malays J Pathol. 2006 Jun; 28(1): 7-15.
20. Hollants S, Redeker EJ, Matthijs G. Microfluidic Amplification as a Tool for Massive Parallel Sequencing of Familial Hypercholesterolemia Genes. Clin Chem. 2012 Jan 31.
21. Chiou KR, Charng MJ, Chang HM. Array-based resequencing for mutations causing familial hypercholesterolemia. Atherosclerosis. 2011 Jun; 216(2): 383-9.