DETECTION OF LOW DENSITY LIPOPROTEIN RECEPTOR GENE MUTATIONS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLAEMIA

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ABSTRACT

Familial hypercholesterolaemia (FH) is an autosomal dominant inherited disorder of lipoprotein metabolism associated with premature coronary artery disease (CAD). Extrapolating the prevalence of heterozygous FH in western populations into Malaysian population, it would be estimated that about 50,000 Malaysians would be affected and at risk of developing premature CAD. Mutations of the low density lipoprotein receptor (LDLR) gene are the most frequent cause of FH but its genetic profiles in the Asian population has been poorly characterised. Therefore, in this study, the screening and characterisation of the LDLR gene mutations and its polymorphisms among patients with clinical diagnosis of FH were compared to normocholesterolaemic controls. Cross sectional study involving 74 FH patients (50 Malays & 24 Chinese) and 77 age-matched normocholesterolaemic controls were recruited. Diagnosis of FH was based on Simon Broome Register criteria. Blood samples were collected, serum and plasma were separated, stored and analysed for fasting serum lipids, glucose, renal profile, liver function tests and thyroid function test by standard automated laboratory techniques. Whole blood in EDTA tubes were collected and stored in -80 ^{0}C until DNA extractions were performed using a commercial kit. Genomic DNA purity was determined using a spectrophotometer. Gene amplification by polymerase chain reaction (PCR) was employed to amplify all exons (exon 1 – 18) and the promoter region of the LDLR gene. Mutation screening analysis was performed by denaturing high performance liquid chromatography (DHPLC). Samples showing heteroduplexes profiles by DHPLC were sequenced to confirm the location and nature of mutations using Sanger sequencing method. The present study detected five LDLR variants among 46 (62.2%) of FH patients in intron 3, exon 5, 9, 10 and 12 using DHPLC. Three different types of LDLR mutations were confirmed by Sanger sequencing in 6 out of 46 (13%) clinically-diagnosed FH patients. The identified mutations were in intron 3 (g.313+1G>A) of a FH patient of Chinese ethnicity, exon 5 (g.763T>A; C234S) of four FH patients of a Malay family and exon 9 (g.1216C>T; R385W) of a FH patient of Chinese ethnicity. Of the identified Malay family members, a homozygote and three heterozygotes for LDLR C234S mutation were found. Other variants identified were in exons 10 (g.1413G>A) and 12 (g.1773T>C). However, both variants in exons 10 and 12 did not cause amino acid change (arginine (R) at codon 450 [AGG>AGA] and proline (N) at codon 570 [AAT>AAC]) respectively. In conclusion, the present work of employing molecular screening using DHPLC has been successfully optimised to detect the LDLR gene mutations. Combined with the clinical diagnosis criteria, the use of DNA-based screening method plays an important role in the definitive diagnosis of FH.

ABSTRAK

Hiperkolesterolemia familial (FH) adalah penyakit metabolisma lipoprotin keturunan yang diwariskan secara autosomal dominan dan mengaruh kepada peningkatan risiko penyakit arteri koronari pramatang. Menggunakan prevelan pada populasi di negara-negara Barat, dianggarkan seramai 50,000 penduduk Malaysia menghidap penyakit FH dan mempunyai risiko penyakit arteri koronari pramatang. Mutasi pada gen reseptor lipoprotin berketumpatan rendah (LDLR) merupakan punca utama penyakit FH. Walaubagaimanapun, pengenalpastian profil genetik penyakit ini di negara-negara Asia adalah kurang meluas. Kajian hirisan lintang ini dijalankan bagi menyaring dan mengenalpasti mutasi gen LDLR di kalangan 74 pesakit FH berbanding 77 subjek yang mempunyai aras kolesterol yang normal. Diagnosis penyakit FH dibuat berdasarkan kriteria Simon Broome. Sampel darah telah diambil untuk ujian aras kolesterol, aras glukosa, fungsi buah pinggang, fungsi hepar, fungsi kelenjar tiroid dan pengekstrakan asid deoksiribonukleik (DNA). Pengekstrakan DNA dari sampel darah dibuat dengan menggunakan kit pengekstrakan DNA komersial dan ketulenannya diukur menggunakan spektrofotometer. Amplifikasi kesemua ekson dan kawasan promoter pada gen LDLR dibuat menggunakan reaksi rantaian polimerase (PCR). Penyaringan mutasi gen LDLR dibuat menggunakan teknik *denaturing high performance liquid chromatography* (DHPLC). Penjujukan DNA dilakukan terhadap sampel-sampel DNA yang menunjukkan profil *heteroduplexes* semasa penyaringan menggunakan DHPLC bagi mengesahkan kehadiran dan jenis mutasi. Menggunakan teknik DHPLC, lima jenis variasi gen LDLR telah dikenalpasti di kalangan 46 (62.2%) pesakit FH iaitu terletak di kawasan intron 3, ekson 5,9,10 dan 12. Tiga jenis mutasi telah disahkan melalui teknik penjujukan DNA pada 6 daripada 46 (13%) pesakit FH. Mutasi yang dikenalpasti adalah di kawasan intron 3 (g.313+1G>A) pada seorang pesakit bangsa Cina, mutasi pada ekson 5 (g.763T>A; C234S) di kalangan empat orang ahli keluarga bangsa Melayu dan mutasi pada ekson 9 (g.1216C>T; R385W) pada seorang pesakit bangsa Cina. Di kalangan empat orang ahli keluarga yang mempunyai mutasi pada ekson 5, seorang adalah homozigot dan tiga orang lagi merupakan heterozigot. Variasi gene LDLR lain melibatkan ekson 10 dan ekson 12 tetapi variasi ini tidak menyebabkan perubahan di peringkat asid amino. Sebagai kesimpulan, teknik DHPLC telah berjaya dibangunkan secara optimum sebagai kaedah saringan mutasi gen LDLR pada pesakit FH. Teknik analisa DNA memainkan peranan penting dalam pengenalpastian jenis mutasi pada penyakit FH disamping diagnosis menggunakan ciri-ciri klinikal.

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LIST OF ABBREVIATIONS

APOB	Apolipoprotein B-100
BP	Blood pressure
bp	base pair
BMI	Body mass index
cDNA	Complementary DNA
CVD	Cardiovascular disease
CAD	Coronary artery disease
CETP	Cholesteryl ester transfer protein
DBP	Diastolic blood pressure
DGGE	Denaturing gradient gel electrophoresis
DHPLC	Denaturing high performance liquid chromatography
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
ddNTPs	Dideoxynucleotide triphosphates
EDTA	Ethylenediaminetetraacetic acid
FH	Familial hypercholesterolaemia
gDNA	Genomic DNA
HDL-c	High density lipoprotein
HMG-CoA	3-Hydroxy-3-methyl-glutaryl-CoA
LDL-c	Low density lipoprotein
LDLR	Low density lipoprotein receptor
LPL	Lipoprotein lipase
LRP	LDL-receptor like protein

MLPA	Multiplex ligation-dependent probe amplification
PCSK9	Proprotein convertase subtilisin/kexin type 9
PCR	Polymerase chain reaction
SBP	Systolic blood pressure
SCAP	Sterol cleavage-activating protein
SNPs	Single Nucleotide Polymorphisms
SREBP	Sterol regulatory element binding protein-2
SSCP	Single strand conformation polymorphism
TC	Total cholesterol
TG	Triglycerides
T_m	Melting Temperature
VLDL	Very low density lipoprotein
WHR	Waist-to-hip ratio