APPENDIX A - SIMON BROOME REGISTER CRITERIA FOR DIAGNOSIS OF FAMILIAH HYPERCHOLESTEROLAEMIA

DEFINITE FH	POSSIBLE FH
1. Biochemistry : Total cholesterol >7.5mmol/L or LDL cholesterol >4.9mmol/l (aged $\geq$ 16 years) Total cholesterol >6.5mmol/L (aged < 16 years) 2. Tendon xanthomata in $\geq$ 1 family members i.e. either the patient or parents, children, siblings, uncles, aunts or grandparents	1. Biochemistry (as previously stated) **PLUS** Family history of premature CAD in first degree relatives or second degree relatives **OR** 2. Biochemistry (as previously stated) **PLUS** Family history of severe hypercolesterolaemia (TC>7.5mmol/L) in first or second degree relatives.

APPENDIX B- PATIENT CONSENT FORM

Participants Information and Consent Form

Characterization of low density lipoprotein receptor (LDLR) gene in patients with Familial Hypercholesterolaemia in Malaysia

Introduction

You are invited to take part voluntarily in a research study

Familial hypercholesterolaemia (FH*)*

A common inherited disorder with an autosomal dominant pattern of inheritance, associated with premature vascular disease including coronary artery disease leading to significant morbidity and mortality. FH may be caused by different mutations of the low density lipoprotein receptor (LDLR) or its ligand apolipoprotein (Apo) B100 gene.

LDL receptor

The receptor found on cell membranes to which LDL binds to, and gets internalised, hence important in the transport of cholesterol from blood into cells, and regulation of blood cholesterol levels.

Apolipoprotein B100

A component of the LDL which serves as a ligand for the binding of LDL to LDL receptor.

Before agreeing to participate in this research study, it is important that you read and understand this form. It describes the purpose, procedures, benefits, risks, discomforts, and precautions of the study. It also describes the alternative procedures that are available to you and your right to withdraw from the study at anytime. If you participate, you will receive a copy of this form to keep for your records.

Purpose of the Study

1. To screen for low density lipoprotein receptor (LDLR) and gene among patients with familial hypercholesterolaemia in Malaysia
2. To characterize the types of mutations in the LDLR gene among these patients.

It is possible that information collected during this study will be analyzed in the future for other possible uses or other medical or scientific purposes other than those currently proposed.

Qualification to Participate

The doctor in charge of this study or a member of the study staff will discuss with you the requirements for participation in this study. It is important that you are completely truthful with the doctor and staff about you health history. You should not participate in this study if you do not meet all qualifications.

Some of the requirements to be in this study are –

You can participate if:

Diagnosed with FH by fullfilling the the Simon Broome's criteria. The criteria are as follows:

DEFINITE FH	POSSIBLE FH
1. Biochemistry : Total cholesterol > 7.5 mmol/L or LDL cholesterol > 4.9 mmol/L [age ≥ 16 yrs] Total cholesterol>6.5 mmol/L [aged < 16 yrs] 2. Tendon xanthomata in ≥ 1 family members i.e. either in the patient or parents, children, siblings, uncles, aunts or grandparents.	1. Biochemistry (as previously stated) **Plus** Family history of premature CAD in first-degree relatives (<60 yrs) or second-degree relatives (<50 yrs). **OR** 2. Biochemistry (as previously stated) **Plus** Family history of severe hypercholesterolaemia (TC > 7.5 mmol/L) in first or second degree relatives.

- You are able to understand the procedures of the research and to follow simple instruction
- You are willing to sign written informed consent form
- You agree to use the study medicine only as instructed by your study doctor and staff, and to return any unused study medicine and containers at the end of the study or as otherwise instructed by the study doctor.

You cannot participate if:

- Secondary hyperlipidaemia eg hypothyroidism, renal (serum creatinine >110umol/L), liver diseases
- You have previously participated in or withdraw from such study
- You are not able to understand the procedures of the research and to follow simple instructions
- You are not willing to sign written informed consent form

Study Procedures

1. This is a crossectional study.
2. For each subject, a set of questionnaire will be completed and a detailed history taking and physical examination will be performed to obtain the relevant clinical data.
3. Family tracing and screening will be performed for each proband
4. Normolipaemic controls from the three major ethnic groups will be recruited in parallel
5. Baseline ECG, exercise tolerance test, endothelial function and carotid artery intima-media thickness will be performed on all subjects.
6. Twelve hours-(12) hours fasting blood samples and urine of clinically diagnosed individuals, their family members and controls will be collected, separated, stored and analysed as appropriate**.**
7. Serum samples will be analysed for several baseline biochemical tests – eg fasting serum lipids, glucose, renal profile, liver

function tests and urinalysis by automated standard laboratory techniques.

8. DNA extraction will be performed using commercial kits and the purity will be determined by using a spectrophotometer.
9. Mutational screening will be done by the DHPLC technique, with positive and negative controls being run in parallel
10. Samples with positive mutational screening results will be subjected to DNA sequencing for confirmation of the mutations

Following the study, the study doctor or their representatives may contact you to obtain further information regarding your experiences if needed.

Risks

This study is only a cross sectional study. Patients will not be involved with any drugs trial.

Reporting Health Experiences

Please contact Prof. Dr. Hapizah Mohd. Nawawi, Faculty of Medicine,
Universiti Teknologi Mara, Shah Alam, Selangor (Telephone no: 03-55442841)

Participation in the Study

Your taking part in this study is entirely voluntary. You may refuse to take part in the study or you may stop participation in the study at anytime, without a penalty or loss of benefits to which you are otherwise entitled. Your participation also may be stopped by the study doctor or researchers without your consent. If you stop being part of this study, the study doctor or one of the staff member will talk to you about medical issues regarding the stopping of your participation.

Possible Benefits

Beneficial impact on reducing cost of health care with advancement in early global management of patients with FH and their family members who are at risk of premature coronary heart disease, peripheral vascular diseases and stroke, in terms of reducing mortality and morbidity

Investigator Payment

Consultation, blood tests and clinical tests fees are free of charge.

Questions

For futher enquiry, please contact Prof. Dr. Hapizah Mohd. Nawawi, Faculty of Medicine , Universiti Teknologi Mara , Shah Alam, Selangor (Telephone no: 03-55442830,)

Confidentialty

Your medical information will be kept confidential by the study doctor and staff and will not be made publicly available unless disclosure is required by law.

The information obtained from this study will be saved in a Data Bank and processed with a computer. Data obtained from this study that does not identify you individually and may be used for further analysis and published internationally or given to the government.

By signing this consent form, you authorize the record review, information storage and data transfer.

Signatures

To be entered into the study, you or a legal representative must sign and data the signature page (see Attachment 1)

Signature Page **Attachment 1**

To become a part this study, you or your legal representative must sign this page.

Consent Form

Characterization of low density lipoprotein receptor (LDLR) gene in patients with Familial Hypercholesterolaemia in Malaysia

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By signing this page, you are confirming the following:

1. You have read all of the information in this Patient Informaiton and Consent Form, and you have had time to think about it.
2. All of your questions have been answered to your satisfaction.
3. You voluntarily agree to be part of this research study, to follow the study procedures, and to provide necessary information to the doctor, nurses, or other staff members, as requested.
4. You voluntarily allow your urine and blood samples including DNA to be used for the purpose of this study.
5. You may freely choose to stop being a part of this study at anytime.
6. You have received a copy of this Patient Information and Consent Form to keep for yourself.

Name of participant :_____ Name of Witness :_____

I/C No: _____ I/C No:_____

Participant Initials and Number : _____

_____ _____

Signature of participant or Signature of Witness

Legal Representative Date (dd/mm/yy)

Date (dd/mm/yy) (add time of day if appropriate)

(add time of day if appropriate)

Doctor responsible in obtaining the inform consent:

Name of Doctor:_____

Identity Card Number:_____

_____

Signature of Doctor
Date (ddMMyy) (add time of day if appropriate)

Borang Makluman Pesakit dan Borang Persetujuan (versi Bahasa Melayu)

Pengenalpastian mutasi gen reseptor lipoprotin berketumpatan rendah pada pesakit Hiperkolesterolemia Familial

Latarbelakang kajian:

Hyperkolesterolemia Familial (FH*)*

Merupakan penyakit metabolisme lipoprotin keturusan yand diwariskan secara autosomal dominan dan mengaruh kepada peningkatan risiko penyakit jantung pramatang. Penyakit ini boleh disebabkan oleh beberapa jenis mutasi pada gen reseptor lipoprotin berketumpatan rendah (LDLR) atau gen ligan apolipoprotein B100 (ApoB).

Reseptor LDL

Reseptor ini terletak di membrane sel dimana lipoprotin berketumpatan rendah akan mengikat kepadanya and seterusnya dapat mengangkut kolesterol dari darah ke dalam sel and mengawalatur aras kolesterol di dalam darah.

Apolipoprotein B100

Merupakan komponen lipoprotin berketumpatan rendah yang bertindak sebagai tapak ligan untuk pengikatan lipoprotin berketumpatan rendah kepada reseptornya.

Tujuan Kajian :

1. Melakukan penyaringan mutasi gen LDLR pada pesakit FH di Malaysia.
2. Mencirikan jenis-jenis mutasi gen LDLR dikalangan pesakit FH.

Terdapat kemungkinan maklumat yang dikumpulkan semasa kajian ini akan dianalisakan pada masa depan untuk kegunaan lain yang mungkin atau untuk tujuan perubatan atau saintifik lain yang selain dari yang kini dicadangkan.

Kelayakan Penyertaan

Doktor yang bertanggungjawab dalam kajian ini atau salah seorang kakitangan kajian akan membincangkan kelayakan untuk menyertai kajian ini dengan anda. Adalah penting anda berterus terang dengan doktor dan kakitangan tersebut tentang sejarah kesihatan anda. Anda tidak seharusnya menyertai kajian ini sekiranya anda tidak memenuhi semua syarat kelayakan.

Beberapa keperluan untuk menyertai kajian ini adalah –:

Anda hanya boleh menyertai kajian ini sekiranya:

(a) Anda di diagnosa sebagai pesakit hiperkolesterolemia keturunan berdasarkan kriteria Simon Broome's. Kriteria Simon Broome's adalah seperti berikut:

DEFINITE FH	POSSIBLE FH
1. Ciri – ciri biokimia • Kolesterol jumlah > 7.5 mmol/L /LDL > 4.9 mmol/L bagi pesakit berumur ≥ 16 yrs • Kolesterol jumlah > 6.5 mmol/L bagi pesakit berumur < 16 yrs **DAN** 2.Kehadiran tendon xantomata pada pesakit atau ≥ 1 ahli keluarga i.e. ibubapa, anak, adik beradik, bapa saudara, ibu saudara, datuk atau nenek.	1. Ciri – ciri biokimia (seperti Definite FH) **DAN** sejarah penyakit arteri koronari pramatang dikalangan ahli keluarga peringkat pertama (<60 tahun) atau ahli keluarga peringkat kedua (<50 yrs). **ATAU** 2. Ciri – ciri biokimia (seperti Definite FH) **DAN** Sejarah keluarga mengidap hiperkolesterolemia yang (TC > 7.5 mmol/L) dikalangan ahli keluarga peringkat pertama dan kedua.

(b) Anda boleh memahami protokol kajian ini dan mengikuti arahan yang senang

(c) Anda bersetuju mangambil bahagian dalam kajian ini dan menandatangani borang persetujuan ini.

Anda tidak boleh menyertai kajian ini sekiranya –:

- Sebelum ini anda pernah menyempurnakan atau menarik diri dari kajian ini
- Anda tidak boleh memahami protocol kajian ini dan tidak boleh mengikuti arahan yang senang
- Anda tidak bersetuju untuk menandatangani borang persetujuan ini
- Anda bersetuju mengikuti kajian hanya seperti yang diarahkan oleh doktor dan kakitangan kajian, dan memulangkan sebarang ubat dan bekas kajian yang tidak digunakan pada akhir kajian atau seperti yang dimaklumkan oleh doktor kajian.

Prosedur-prosedur Kajian

1. Kajian ini merupakan kajian hirisan melintang.
2. Buku soal selidik akan disediakan untuk setiap subjek kajian. Data sejarah pesakit, data pengukuran antropometri dan data pemeriksaan fizikal akan dilengkapkan.
3. Penyaringan mutasi setiap ahli keluarga pesakit akan turut dilakukan.
4. Setiap subjek akan menjalani ujian fungsi endothelial, ujian senaman, ujian pengukuran ketebalan lapisan tunika intima - media pada saluran darah karotid dan ujian ‘eletrocardiograpgh’ (ECG).
5. Sampel darah dan urin subjek akan diambil selepas subjek diminta berpuasa selama 12 jam.
6. Sampel darah bagi tujuan pengektrakan asid deoksiribonukleik (DNA) akan disimpan pada suhu 80^{o}C untuk memastikan kestabilannya.
7. Plasma dan serum setiap subjek turut dikumpulkan dan disimpan dalam suhu - 80^{o}C untuk memastikan kestabilannya.

8. Pengekstrakan DNA dilakukan dengan menggunakan kit yang telah dikomersialkan. Kepekatan DNA ditentukan dengan spektrofotometer.
9. Pengesanan mutasi LDLR dan Apo B100 akan dilakukan menggunakan 'Denaturing High Performance Liquid chromatography' (DHPLC).
10. Sampel yang menunjukkan keputusan positif pada DHPLC akan dilakukan penjujukan DNA.

Selepas kajian, doktor kajian, pihak penaja kajian atau wakil mereka mungkin akan menghubungi anda untuk mendapatkan maklumat lanjut sekiranya perlu.

Risiko

Pesakit yang menyertai kajian ini tidak akan menghadapi sebarang risiko kerana kajian ini tidak melibatkan sebarang pemberian ubat-ubatan dan tidak melibatkan prosedur pemeriksaan yang menyakitkan.

Melaporkan Pengalaman Kesihatan

Sekiranya terdapat sebarang masalah sila hubungi Prof. Dr. Hapizah Mohd. Nawawi, no telefon – 03-55442841.

Penyertaan Dalam Kajian

Penyertaan anda dalam kajian ini adalah secara sukarela. Anda boleh menolak penyertaan dalam kajian ini atau anda boleh menamatkan penyertaan anda dalam kajian ini pada bila-bila masa, tanpa sebarang hukuman atau kehilangan sebarang manfaat yang sepatutnya diperolehi oleh anda.

Penyertaan anda mungkin juga diberhentikan oleh doktor kajian atau pihak penyelidik tanpa persetujuan anda.

Jika anda berhenti menyertai kajian ini, doktor kajian atau salah seorang kakitangan akan berbincang dengan anda mengenai apa-apa isu perubatan berkenaan dengan pemberhentian penyertaan anda.

Manfaat yang Mungkin

Kajian ini penting untuk pengurusan pesakit yang mengidap hiperkolesterolemia keturunan dimana pengesanan awal mutasi LDLR dan Apo B100 pada pesakit ini dapat membantu mereka merendahkan faktor risiko mendapat penyakit arteri koronari dan data ini dapat menyumbang kepada pembangunan rawatan menggunakan gen terapi pada masa akan datang.

Bayaran Doktor (Penyelidikan)

Kesemua kos rundingan dan ujian darah adalah percuma.

Soalan

Jika pesakit mempunyai sebarang pertanyaan, sila hubungi Prof. Dr. Hapizah Mohd Nawawi, Fakulti Perubatan, Universiti Teknologi Mara, Shah Alam, Selangor (Tel: 03- 55442841)

Kerahsiaan

Maklumat perubatan anda akan dirahsiakan oleh doktor dan kakitangan kajian dan tidak akan dedahkan secara umum melainkan jika ia dikehendaki oleh undang-undang.

Maklumat perubatan anda mungkin akan disimpan dalam komputer dan diproses dengannya. Data yang diperolehi dari kajian yang tidak mengenalpasti anda secara perseorangan tetapi mungkin dianalisakan kemudian, diterbitkan di peringkat

international atau diberi kepada pihak penyelidik dan/atau wakil-wakilnya atau pihak berkuasa.

Dengan menandatangani borang persetujuan ini, anda membenarkan penelitian rekod, penyimpanan maklumat dan pemindahan data seperti yang dihuraikan diatas.

Tandatangan

Untuk dimasukkan ke dalam kajian ini, anda atau wakil sah anda mesti menandatangani serta menarikhkan halaman tandatangan (lihat Lampiran 1)

Halaman Tandatangan **Lampiran 1**

Untuk menyertai kajian ini, anda atau wakil sah anda mesti menandatangani mukasurat ini.

Borang Maklumat dan Keizinan Peserta

Penyaringan mutasi gene reseptor lipoprotin ketumpatan rendah (LDLR) pada pesakit hiperkolesterolemia keturunan di Malaysia

Dengan menandatangani mukasurat ini, saya mengesahkan yang berikut:

1. Saya telah membaca semua maklumat dalam Borang Maklumat dan Keizinan Pesakit ini, dan saya telahpun diberi masa yang mencukupi untuk mempertimbangkan maklumat tersebut.
2. Semua soalan-soalan saya telah dijawab dengan memuaskan
3. Saya, secara sukarela, bersetuju menyertai kajian penyelidikan ini, mematuhi segala prosedur kajian dan memberi maklumat yang diperlukan kepada doktor, para jururawat dan juga kakitangan lain yang berkaitan apabila diminta.
4. Saya membenarkan sample darah, urin dan DNA saya diambil untuk kegunaan kajian ini.
5. Saya boleh menamatkan penyertaan saya dalam kajian ini pada bila-bila masa.
6. Saya telahpun menerima satu salinan Borang Maklumat dan Keizinan Pesakit untuk simpanan peribadi saya.

Nama peserta :

No kad pengenalan :

Nombor dan 'Initial' Peserta

_____

Tandatangan Peserta atau Wakil sah

Tarikh (ddMMyy)

(tambahkan masa jika sesuai)

Nama saksi:

No kad pengenalan:

_____

Tandatangan saksi

Tarikh (ddMMyy)

(tambahkan masa jika sesuai)

Doktor yang bertanggungjawab menjalankan sesi persetujuan ini:

Nama Doktor:

No kad pengenalan:

_____

Tandatangan Doktor

Tarikh (ddMMyy)

(tambahkan masa jika sesuai)

APPENDIX C: PATIENT DATA COLLECTION SHEET

PATIENT DATA COLLECTION SHEET

1. Name :
2. I/C no.:
3. Gender:
4. Age:
5. Race:
6. Diagnosis: ☐ FH ☐ NFH
7. Cardiovascular risk factors:
    a. Smoking: non-smoker ☐ current smoker ☐ ex-smoker
    b. BP:
    c. History of CAD :
        i. No ☐
        ii. Yes: ☐
        (pls specify): _____
        _____
    d. Family history of CAD :
        i. No ☐
        ii. Yes ☐
        (pls Specify): _____
        _____
8. Other past medical history:
    a. Thyroid disease ☐ No ☐ Yes (Specify_____)
    b. Diabetes ☐ No ☐ Yes (Specify_____)
    c. Inflammatory disease ☐ No ☐ Yes (specify_____)
    d. Renal disease ☐ No ☐ Yes (Specify_____)
    e. Liver disease ☐ No ☐ Yes (Specify_____)
    f. Cancer ☐ No ☐ Yes (Specify_____)

9. Medications:
    a. Immunosuppressive therapy: ☐ No ☐ Yes (_____)
    b. Alcohol: ☐ No ☐ Yes
    (_____)
    c. Steroids: ☐ No ☐ Yes
    (_____)
    d. Vitamins: ☐ No ☐ Yes
    (_____)
    e. Lipid lowering mediations: ☐ No ☐ Yes
    (_____)

Appendix D – DHPLC Temperature Profiles

Exon	PCR product size (bp)	Annealing temperature (^{O}C)	DHPLC temperature (^{O}C)
Promoter	**275**	**57.0**	**62.0**
Exon 1	**244**	**57.0**	**56.5**
Exon 2	**189**	**57.0**	**62.6**
Exon 3	**196**	**57.0**	**63.7**
Exon 4-5'	**187**	**57.0**	**64.7**
Exon 4-mid	**186**	**57.0**	**63.7**
Exon 4-3'	**240**	**57.0**	**62.7**
Exon 5	**182**	**57.0**	**62.8**
Exon 6	**180**	**57.0**	**62.6**
Exon 7	**242**	**57.0**	**64.9**
Exon 8	**206**	**57.0**	**64.0**
Exon 9	**275**	**57.0**	**62.6**
Exon 10-5'	**213**	**57.0**	**62.7**
Exon 10-3'	**166**	**57.0**	**62.7**
Exon 11	**177**	**57.0**	**62.0**
Exon 12	**213**	**57.0**	**63.1**
Exon 13	**217**	**57.0**	**59.1**
Exon 14	**284**	**57.0**	**64.2**
Exon 15	**255**	**57.0**	**63.1**
Exon 16	**134**	**65.0**	**64.1**
Exon 17	**245**	**57.0**	**61.6**
Exon 18	**140**	**57.0**	**63.0**

Appendix E

List of Publications

a) Abstracts in proceeding

1. Razali R, Froemming GA, Suhana NH, Hoh BP, Abd Rahman TH, Noraida I, Sahilah AM, and Nawawi H. Characterisation Of Low Density Lipoprotein Receptor Gene In Patients With Familial Hypercholesterolaemia. 35th Australian Atherosclerosis Society Annual Scientific Meeting. Melbourne, Australia. 13 – 16 October 2009.
2. Razali R, Froemming GA, Suhana NH, Hoh BP, Abd Rahman TH, Noraida I, Sahilah AM, and Nawawi H. Low Density Lipoprotien Receptor Gene Mutations in Patients with Familial Hypercholesterolaemia of Malay and Chinese Descent. 7th Asian-Pacific Society of Atherosclerosis and Vascular Disease Congress. Cairns, Australia. 26 – 29 October 20101.

Appendix F

Posters Presentation

CHARACTERISATION OF LOW DENSITY LIPOPROTEIN RECEPTOR GENE IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLAEMIA

Razali R[1], Froemming GA[1], Suhana NH[1], Hoh BP[1], Abd Rahman TH[1], Ismail T[1], Noraida I[2], Sahilah AM[3] and Nawawi H[1].
[1]Faculty of Medicine, Universiti Teknologi MARA, Shah Alam, Malaysia
[2]Faculty of Science, Universiti Malaya AND [3]Faculty of Science &Technology, Universiti Kebangsaan Malaysia.

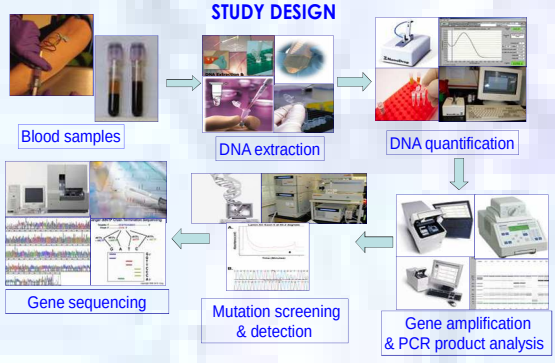
BACKGROUND

- Familial hypercholesterolaemia (FH) is a co-dominant inherited disorder of lipoprotein metabolism associated with premature coronary artery disease.
- Mutations of the low density lipoprotein receptor (LDLR) gene are the most frequent cause of FH but mutations in the Asian population has been poorly characterised
- The estimated heterozygote prevalence in most population is 1:500.
- Population of Malaysia around 27.2 million (2007 est.), estimated about 54000 individuals are affected.
- Diagnosis of FH is currently based on clinical and biochemical criteria rather than identification of disease-causing mutations.

OBJECTIVE

- To characterise the LDLR gene mutations and polymorphism among patients with FH compared to controls.

STUDY DESIGN



DIAGNOSIS OF FH – SIMON BROOM'S CRITERIA

DEFINITE FH	POSSIBLE FH
1. Biochemistry : TC > 7.5mmol/L or LDL-c > 4.9mmol/L (aged ≥16Y) TC > 6.5mmol/L (aged < 16 Y) 2. Tendon xanthomata in ≥ 1 family members ie either the patient or parents, children, siblings, uncles, aunts or grandparents	1. Biochemistry : TC > 7.5mmol/L or LDL-c > 4.9mmol/L (aged ≥16Y) TC > 6.5mmol/L (aged < 16 Y) PLUS Family history of premature CAD in first degree relatives or second degree relatives OR 2. Biochemistry (as previously stated) PLUS Family history of severe hypercholesterolaemia (TC>7.5mmol/L) in first or second degree relatives.

PHYSICAL SIGNS OF PATIENTS WITH FH

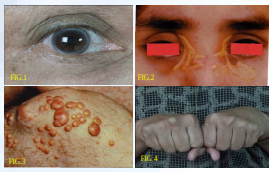


FIGURE 1 : Corneal arcus (Grade 4) FIGURE 2 : Xanthelasma
FIGURE 3 : Knee xanthomata FIGURE 4 : Tendon xanthomata on the hand

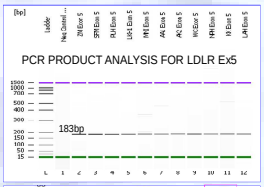
RESULTS

BASELINE CHARACTERISTICS OF SUBJECTS

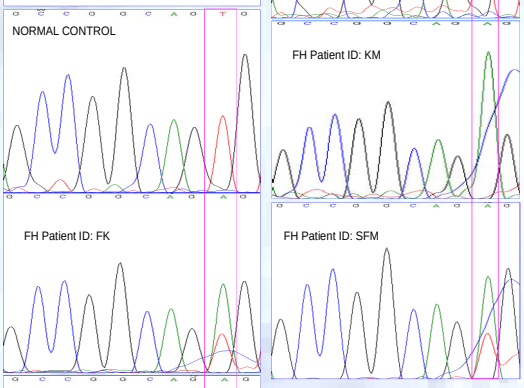
PARAMETERS	FH (n=96)	Control (n=79)
[b]Age (years)	44.2 ± 12.2	46.3 ± 10.3
[a] Gender (M/F)	37/59 (38.5% / 61.5%)	26/53 (32.9% / 67.1%)
[a]Current smoker (%)	14.7%	10.4%
[b]BMI (kg/m2)	24.5 ± 4.7 *	23.0 ± 4.6
[b]WHR	0.84 ± 0.07 *	0.81 ± 0.06
[b]TC (mmol/L)	8.5 ± 1.7*	5.2 ± 0.9
[b]TG (mmol/L)	1.9 ± 1.7*	1.1 ± 0.5
[b]LDL-c (mmol/L	6.4± 1.6*	3.2 ± 0.8
[b]HDL-c (mmol/L)	1.3 ± 0.3*	1.4 ± 0.3

*p<0.05 compared to control;
[a]Data expressed expressed as proportion (percentage), [b]Data as mean ± SD.

PCR Product analysis by capillary gel electrophoresis (Agilent Bioanalyser)



Sequencing result by DNA sequencer (Applied Biosystem DNA sequencer)



CONCLUSION

Mutation C234S (substitution T>A) in exon 5 of LDLR gene has been identified and characterised in a Malay family clinically diagnosed as FH.

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LOW DENSITY LIPOPROTEIN RECEPTOR GENE MUTATIONS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLAEMIA OF MALAY AND CHINESE DESCENT

Razali R[1], Froemming GA[1], Suhana NH[1], Hoh BP[1], Abd Rahman TH[1], Noraida I[2], Sahilah AM[3] and Nawawi H[1].
[1]Faculty of Medicine, Universiti Teknologi MARA, Shah Alam, Malaysia
[2]Faculty of Science, Universiti Malaya AND [3]Faculty of Science & Technology, Universiti Kebangsaan Malaysia.

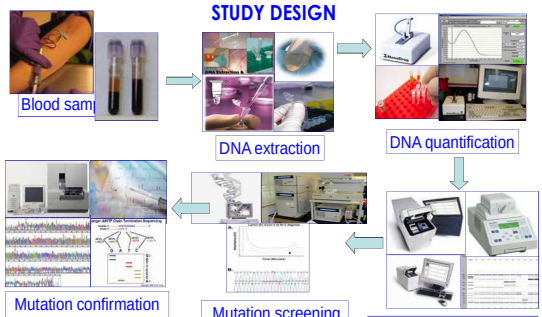
BACKGROUND

- Familial hypercholesterolaemia (FH) is a co-dominant inherited disorder of lipoprotein metabolism associated with premature coronary artery disease.
- 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
- The estimated heterozygote prevalence in most population is 1:500.
- Population of Malaysia around 27.2 million (2007 est.), estimated about 54000 individuals are affected.
- Diagnosis of FH is currently based on clinical and biochemical criteria rather than identification of disease-causing mutations.

OBJECTIVE

- To characterise the LDLR gene mutations and polymorphism among patients with FH compared to controls.

STUDY DESIGN



DIAGNOSIS OF FH – SIMON BROOME CRITERIA

DEFINITE FH	POSSIBLE FH
1. Biochemistry : TC > 7.5mmol/L or LDL-c > 4.9mmol/L (aged ≥16Y) TC > 6.5mmol/L (aged < 16 Y) 2. Tendon xanthomata in ≥ 1 family members ie either the patient or parents, children, siblings, uncles, aunts or grandparents	1. Biochemistry : TC > 7.5mmol/L or LDL-c > 4.9mmol/L (aged ≥16Y) TC > 6.5mmol/L (aged < 16 Y) PLUS Family history of premature CAD in first degree relatives or second degree relatives OR 2. Biochemistry (as previously stated) PLUS Family history of severe hypercholesterolaemia (TC>7.5mmol/L) in first or second degree relatives.

PHYSICAL SIGNS OF PATIENTS WITH FH

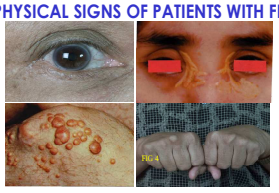


FIGURE 1 : Corneal arcus (Grade 4) FIGURE 2 : Xanthelasma
FIGURE 3 : Knee xanthomata FIGURE 4 : Tendon xanthomata on the hand

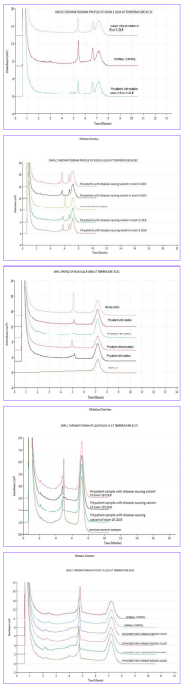
RESULTS

BASELINE CHARACTERISTICS OF SUBJECTS

PARAMETERS	FH (n=74)	Control (n=76)	p value
[b]Age (years)	45.9 ± 12.0	46.3 ± 10.3	NS
[a] Gender (M/F)	28/49 (36.8% / 63.6%)	26/53 (32.9% / 67.1%)	NS
[a]Current smoker (%)	14.5%	17.4%	NS
[b]BMI (kg/m2)	24.3 ± 4.67*	23.0 ± 4.6	NS
[b]WHR	0.84 ± 0.07 *	0.81 ± 0.06	NS
[b]TC (mmol/L)	8.6 ± 1.7*	5.2 ± 0.9	p<0.0001
[b]TG (mmol/L)	2.0 ± 1.8*	1.1 ± 0.5	p<0.0001
[b]LDL-c (mmol/L	6.4± 1.6*	3.2 ± 0.8	p<0.0001
[b]HDL-c (mmol/L)	1.3 ± 0.4*	1.4 ± 0.3	p<0.0001

*p<0.05 compared to control; NS = not signficant
[a]Data expressed expressed as proportion (percentage), [b]Data as mean ± SD.

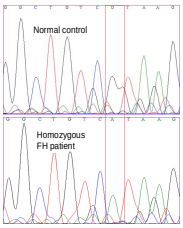
Mutation Screening Result by DHPLC



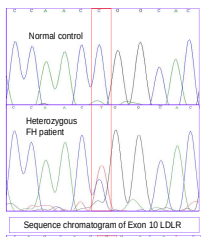
Mutational Confirmation by Sequencing

No	LDLR regions	DNA sequence	Description of variant	No of patient affected & ethnicity
1	Intron 3	Normal : GCAAGCTCTGTCGTAAGT Variant : GCAAGCTCTGTCATAAGT	313+1G>A	1, Chinese
2	Exon 5	Normal : GCCGGCAGCGTGACCG Variant : GCCGGCAGCATGACCG	763T>A, C234S	4, Malays
3	Exon 9	Normal : CTTCACCAACGGGCACG Variant : CTTCACCAACTGGCACG	1216C>T, R385W	1, Chinese
4	Exon 10	Normal : CATCAGCCACGACATCC Variant : CATCAGCAGGACATCC	1413G>A	23, Chinese (5) & Malay (18)
5	Exon 12	Normal : CGATGTCAATGGGGGCA Variant : CGATGTCAAGGGGGCA	1773T>C	23, Chinese (9) & Malay (14)

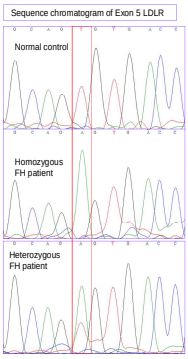
Sequence Chromatogram of Exon 3 LDLR



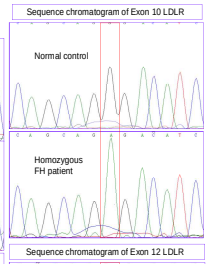
Sequence Chromatogram of Exon 9 LDLR



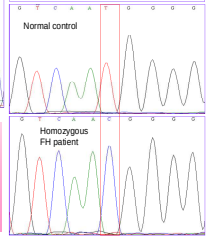
Sequence chromatogram of Exon 5 LDLR



Sequence chromatogram of Exon 10 LDLR



Sequence chromatogram of Exon 12 LDLR



CONCLUSION

Mutation in exon 5 (C234S) of LDLR gene has been identified and characterised in four patients of a Malay family clinically diagnosed as FH while mutations in intron 3 (313+1G>A) and exon 9 (R385W) were identified in two patients of Chinese descent.

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