Single Nucleotide Polymorphisms in Low-Density Lipoprotein Receptor (LDLR) and Upstream Transcription Factor 1 (USF1) Genes Associated with Familial Hypercholesterolaemia among Iban and Bidayuh Ethnic Groups in Sarawak

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DECLARATION

I declare that the work on this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. It is original and is the result of my work, unless otherwise indicated or acknowledged as referenced work. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Familial Hypercholesterolaemia (FH) is a genetic disease caused by defects in a number of genes and variants that regulate plasma LDL-cholesterol concentrations. Insofar no study has been undertaken to determine the frequencies of lipid related gene in Sarawak. We aimed to determine the distribution of LDLR and USF1 gene-associated FH in Iban and Bidayuh ethnic groups in Sarawak, and its associations with lipid profiles. A total of 255 Iban and Bidayuh were recruited. Physical assessments were performed, and two blood tubes were withdrawn. Subjects’ clinical parameters were tested, and DNA was extracted. Allele Specific-PCR of LDLR and USF1 was performed for six single nucleotide polymorphisms (SNPs) and categorised into homozygous wild, heterozygous and homozygous SNP. The data were analysed for genotype-lipid level associations by using the SPSS. FAMHAP with Haploview software was used only for haplotypes analysis and GMDR software used for interpreting the gene-gene interactions towards the LDL risk. Two LDLR gene variants, c.1060+7 T>C variant and c.1706-55A>C variant were detected with majority in homozygous SNP and three genotype stages (homozygote SNP, heterozygote SNP and homozygote wild), respectively. The c.1194C>T variant is not found in this study. Three variants: g.12498165G>A variant, g.7637G>A variant and 306A>G variant in USF1 gene were detected with three genotype stages. The allele and the minor allele frequency (MAF) are different in both studied genes. For c.1060+7 T>C variant and c.1706-55A>C variant, the minor C allele that was found became the major allele with the frequency of 0.996 and 0.535, respectively. There are differences in MAF allele for g.7637G>A variant and 306A>G variant, where the A allele as the wild type and C allele as minor allele, respectively. Result from the current study did not provide any
suggestive evidence of SNP-lipid association in single-SNP model and multi-SNP models to investigate the FH gene of predisposition to hypercholesterolemia. Except, for the two best models of gene-gene interactions of bi-link for g.12498165G>A and 306A>G ($p = 0.003$) and tri-link for c. 1706-55A>C, g.12498165G>A and 306A>G ($p = 0.000$). This study clearly demonstrates that these variants in FH gene have different phenotypes expression, including the candidates with normal LDL level or homozygous. Taken together all the variables into consideration, there is no suggestive significant for the SNP-LDL levels in these two genes. This might be due to the: intronic factor, non-pathogenic factor and linkage equilibrium values are weak in both these genes. More importantly, the peculiar ethnicity of the Iban and Bidayuh could be a major contributing factor of variability (in MAF and association with LDL). In addition, further studies are recommended to replicate this finding of strong interaction in gene-lipid risk model for gene-gene interactions (genotype base of LDL-C). Five SNPs of FH were detected but does not support a significant genetic contribution of desired SNPs and haplotypes to hypercholesterolemia, and the findings are inconclusive regarding their contribution to disease-related traits. Nevertheless, this data is the first to demonstrate the FH variant profile in Iban and Bidayuh ethnic groups and ratified with previous findings of differences due to ethnicity factor and multi-phenotype expressions of FH.

**Keywords:** Familial Hypercholesterolemia (FH), Single Nucleotide Polymorphisms (SNPs), *Low-Density Lipoprotein Receptor* Gene (*LDLR*) and *Upstream Transcription Factor 1* Gene (*USF1*), Low Density Lipoprotein concentration (LDL-C).
Gen Receptor Lipoprotein Ketumpatan Rendah (LDLR) dan Gen Transkripsi Faktor 1 Upstream (USF1) berkaitan dengan Polimorfisme Nukleotida Tunggal dalam Gen Lipid Hiperkolesterolemia Familial di kalangan Etnik Iban dan Bidayuh di Sarawak

ABSTRAK


Kata kunci: Hiperkolesterololemia Familia (FH), Polimorfisme nukleotida tunggal (SNPs), Gen Reseptor Lipoprotein Ketumpatan Rendah (LDLR) dan Gen Transkripsi Faktor 1 Upstream (USF1), kadar Lipoprotein Ketumpatan Rendah (LDL-C).
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<td>Figure 3.2</td>
<td>Representative electrophoresis gel for c. 1706-55A&gt;C variant of \textit{LDLR}.</td>
<td>75</td>
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<td>Figure 3.3</td>
<td>Representative electrophoresis gel for c.1194C&gt;T variant of \textit{LDLR}.</td>
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<tr>
<td>Figure 3.4</td>
<td>Representative electrophoresis gel for g.12498165G&gt;A variant of \textit{USF1}.</td>
<td>76</td>
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</table>
Figure 3.5  Representative electrophoresis gel for g.7637G>A variant of USF1.

Figure 3.6  Representative electrophoresis gel for 306A>G variant of USF1.

Figure 3.7  LDLR linkage disequilibrium map in Iban & Bidayuh population.

Figure 3.8  USF1 linkage disequilibrium map in Iban & Bidayuh population.

Figure 3.9  Gene-gene interactions of high-risk genotypes (best model for two-SNPs).

Figure 3.10 Gene-gene interactions of high-risk genotype (best model for three-SNPs).

Figure 3.11 Interaction Fruchterman-Rheingold model from MDR combined attribute network.