Association of Upstream Transcription Factor 1 Gene (*USF1*) rs3737787 with Reduced LDL (low-density lipoprotein) Cholesterol Level among Iban Ethnic Groups in Sarawak Population

Mohd Aminudin Mustapha[1)], Sai-Peng Sim[2)], Hafizah Hanis Hood[2)], Siaw Yun Ted[2)]

ABSTRACT

Objective: **to determine the polymorphic allele and genotype frequencies of *USF1* rs3737787. It aimed to elucidate the association of the polymorphic allele and genotypes with clinical profiles such as total cholesterol (TC), high- density lipoprotein (HDL), low- density lipoprotein (LDL) and homocysteine level in Iban ethnic group in Sarawak.**

Materials and Methods: **One hundred and fifteen (115) individuals of the Iban ethnic group were recruited as the study subjects. The Allele Specific PCR (AS-PCR) was used in the genotyping. Association of genotype frequencies and clinical profile was assessed using One Way ANOVA. As for the association of allele frequencies and clinical profile, Independent Sample T test was used.**

Results: **Genotype frequency showed statistical significant difference between genotypes of Upstream Transcription Factor 1 (*USF 1*) rs3737787 with low-density lipoprotein (LDL) level with p value of 0.035. The wildtype and heterozygous genotypes of *USF 1* rs3737787 is significantly associated with low level of LDL with $F(1,113) = 4.551$, $p < 0.05$.**

Conclusion: **Our results show that the genetic diversity of *USF1* rs3737787 influences the susceptibility to decreased level of LDL in the Iban ethnic group of the Malaysian population.**

KEY WORDS

Upstream Transcription Factor 1, low- density lipoprotein, Iban, Sarawak

INTRODUCTION

The upstream transcription factors 1 (*USF 1*) and *USF 2* are members of the basic helix-loop-helix/leucine zipper transcription factor family (Yamanaka *et al*., 2016). In addition, USFs have been shown to regulate the expression of genes for fatty acid synthesis and insulin signaling, suggesting their involvement in glucide/lipid metabolism (Corre & Galibert, 2005). The *USF1* gene is located at chromosome 1q22-q23. It consists of 11 exons and extends to 6.73 kb. It was found to be genetically associated with Coronary artery disease (CAD) in Finnish families (Pajukanta *et al*., 2004). USF1 was also found can manifest as hypercholesterolemia and have been shown to predispose to premature cardiovascular diseases (Meng *et al*., 2010).

Familial Hypercholesterolemia (FH) is a genetic disease that is characterized by high levels of low density lipoprotein cholesterol (LDLC) and early cardiovascular disease (CVD). Defects in the Low-Density Lipoprotein (*LDLR*) gene was found to be associated with Familial Hypercholesterolemia (FH) which gave rise to a well-characterized clinical phenotype (Scriver, 2001). The same study suggested that FH is strongly influenced by the genetic background whereby the lipid profile, frequencies of xanthomas and onset as well as severity of cardiovascular disease exhibit great variability in their phenotypic expression. FH was found to be associated with increased risk of coronary disease and premature death (Shivraj & Lye, 2011). Monogenic FH was found to attribute to the defect of LDLR and other genes such as Apolipoprotein B 100 (APOB-100) and Proprotien convertase subtilisin kexin type 9 (PCSK9) gene (Rajih & Al-Talib, 2016). About 4% of FH patients was found to have mutation in the promoter region of *LDLR* gene (Khoo, Van Acker, Tan, & Deslypere, 2000).

Lipoprotein is spherical form and consists of a core of hydrophobic lipids (content cholesterol ester, triglyceride as well as small amounts of other fatty compounds. Cholesterol and other fats are transported through the blood stream in the form of round particles called lipoproteins. Low density lipoprotein (LDL) is the vehicle to transport and deriver the cholesterol to other part of the body to maintain the cellular viability and essential for synthesis steroid hormones in normal condition of LDL catabolism system.

Sarawak is the largest state of Malaysia. The indigenous groups make up about 50% of the total population of 2.6 million people. Iban is the largest indigenous group which comprise of 38% and Bidayuh, second largest after the Iban, make up about 10% of the population (Vasudevan, Fathihah, & Patimah, 2011). The incidence of Iban and Bidayuh with Coronary Vascular Disease (CVD) was higher compared to other ethnic groups in Borneo (Sabah & Sarawak) (Fong *et al*., 2014).

Untreated FH patients have 3-4 times higher risk to develop coronary heart disease, compare to individual without FH (Huijgen, Kindt,

Received on June 22, 2020 and accepted on September 25, 2020

1) Centre for Pre-University Studies, Universiti Malaysia Sarawak
94300 Kota Samarahan, Malaysia
2) Department of Paraclinical Science, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak
94300 Kota Samarahan, Malaysia
Correspondence to: Mohd Aminudin Mustapha
(e-mail: mmaminudin@unimas.my)

Table 1: Genotype frequencies of *USF 1* rs3737787and Clinical data

Clinical Data	*USF 1* rs3737787			
	Wildtype (GG)	Heterozygous (GG)	Total	p value
Total Cholesterol				
< 6.2 mmol/L	72	18		
	90	0.663		
≥ 6.2 mmol/L	19	6	25	
LDL				
< 4.1 mmol/L	85	19	104	**0.035***
≥ 4.1 mmol/L	6	5	11	
HDL				
≥ 1 mmol/L	78	22	100	0.441
< 1 mmol/L	13	2	15	
Triglycerides				
< 2.3 mmol/L	68	20	88	0.376
≥ 2.3 mmol/L	23	4	27	
Homocysteine				
< 15 µmol/L	18	8	26	0.158
≥ 15 µmol/L	73	16	89	

* Statistically significant

Table 2: Allele frequencies of *USF 1* rs3737787 and Clinical data

Clinical Data	*USF 1* rs3737787			
	G	A	Total	p value
Total Cholesterol				
< 6.2 mmol/L	156	17	173	0.599
≥ 6.2 mmol/L	50	7	57	
LDL				
< 4.1 mmol/L	195	21	216	0.165
≥ 4.1 mmol/L	11	3	14	
HDL				
≥ 1 mmol/L	179	21	200	0.933
< 1 mmol/L	27	3	30	
Triglycerides				
< 2.3 mmol/L	157	19	176	0.747
≥ 2.3 mmol/L	49	5	54	
Homocysteine				
< 15 µmol/L	43	8	51	0.164
≥ 15 µmol/L	163	16	179	

Table 3: Association of genotypes frequencies of *USF 1* rs3737787 and Clinical data

		Sum of Squares	df	Mean Square	F	Sig.
Total Cholesterol	Between Groups	.032	1	.032	.187	.667
	Within Groups	19.533	113	.173		
	Total	19.565	114			
LDL	Between Groups	.385	1	.385	4.551	**.035***
	Within Groups	9.563	113	.085		
	Total	9.948	114			
HDL	Between Groups	.067	1	.067	.586	.446
	Within Groups	12.976	113	.115		
	Total	13.043	114			
Triglycerides	Between Groups	.141	1	.141	.775	.381
	Within Groups	20.520	113	.182		
	Total	20.661	114			
Homocysteine	Between Groups	.349	1	.349	1.994	.161
	Within Groups	19.773	113	.175		
	Total	20.122	114			

* Statistically significant

Defesche, & Kastelein, 2012). Although there is relationship between *USF 1* gene and FH but the genetic data are inadequate in Malaysia population and none has been reported in Sarawak. Thus, the current study was conducted to determine the polymorphic allele and genotype frequencies of *USF 1* rs3737787 as well as to elucidate the association of the polymorphic allele and genotypes with clinical profiles such as total cholesterol (TC), high- density lipoprotein (HDL), low- density lipoprotein (LDL) and homocysteine level in Iban ethnic group in Sarawak.

MATERIALS AND METHODS

Participant recruitment

Prior to the blood sampling, the study commenced upon receiving approval from the Research Review Board and Ethnics Committee of Universiti Malaysia Sarawak (UNIMAS). All subjects signed the informed consent agreeing to participate in this research. One hundred and fourteen (114) individuals of the Iban ethnic group in Sarawak functioned as study subjects. Each subject must be fasted for 10 hours prior to blood sampling. Participants must be more than 18 years old.

Inclusion criteria

The inclusion criteria were based on ethnicity of Iban without intermixed marriage among other groups for up to two generations.

Exclusion criteria

The respondents who were still under medication for anti-hypertension, anti-cholesterol and respondents who underwent any major surgery 6 months prior to the study were excluded.

DNA extraction

Peripheral blood samples of 115 individuals of Iban ethnic group were collected in EDTA tubes, after getting written informed consent. The collected samples were stored at -20°C. Genomic DNA was extracted using QIAamp DNA Blood Mini Kit (QIAGEN) and the gene of interest was amplified using appropriate primers.

Genotyping

The allele specific primers were 5'-GGCAAGGCTGTCAGTGCAAG -3' (wildtype) or 5'-GGCAAGGCTGTCAGTGCAAA -3' (variant). The common primer used was 5'- GAAGTTCAGAAGGTGTGTCC -3' (giving a PCR product of 98 bp). The USF 1 g.12498165G > A single nucleotide polymorphism was analyzed using allele specific PCR. PCR was carried out with master mic containing 80 ng DNA template, primer (0.2 uM), 2.0 mM $MgCl_2$, 10x buffer, 10 mM dNTP (0.2) and 1.25 Unit taq DNA polymerase (GoTaq(R) Flexi DNA Polymerase) in a total volume of 25 µl. The PCR products were isolated on 2% agarose gel and visualized with ethidium bromide staining. The *USF 1* g.12498165G > A polymorphic genotypes were categorized into homozygous wild, heterozygous and homozygous variant.

Table 4: Association of allele frequencies of USF rs3737787 and LDL

		t	df	Sig. (2-tailed)	95% Confidence Interval of the Difference	
					Lower	Upper
Total Cholesterol	Equal variances assumed	-.524	228	.601	-.233	.135
	Equal variances not assumed	-.492	27.790	.626	-.253	.155
LDL	Equal variances assumed	-1.388	228	.166	-.173	.030
	Equal variances not assumed	-1.012	25.439	.321	-.217	.074
HDL	Equal variances assumed	.083	228	.934	-.138	.150
	Equal variances not assumed	.083	28.644	.934	-.143	.155
Triglycerides	Equal variances assumed	.322	228	.748	-.151	.210
	Equal variances not assumed	.329	28.973	.744	-.154	.213
Homocyctiene	Equal variances assumed	1.390	228	.166	-.052	.301
	Equal variances not assumed	1.218	26.975	.234	-.085	.335

Statistical Analysis

All genotypes and allele frequencies were calculated. Genotype *USF 1* rs3737787 association with lipid profile and homocysteine level were calculated using One Way ANOVA. As for association of allele of *USF 1* rs3737787 with lipid profile and homocysteine level were calculated using Independent Sample T test.

RESULTS

Genotype and allele frequencies of USF 1 rs3737787 and clinical data

On comparing genotype frequencies of *USF1* rs3737787 with clinical data (Table 1), *USF1* rs3737787 genotype shows statistically significant difference in LDL with p value of 0.035.

Table 2 compares the allele frequencies of *USF1* rs3737787 with clinical data, there are no data shows statistically significant difference in all analysis involves.

Association of genotype and allele frequencies of *USF 1* 306 G > A with Clinical Data.

An analysis of variance showed that, homozygous and heterozygous genotype of USF 1 rs3737787 significantly associated with the level of LDL, $F(1,113) = 4.551$, $p < 0.05$ as shown in Table 3.

For allele frequencies analysis, there are no significantly association observed.

DISCUSSION

Studies found that relevant relationship between the USF1 and lipid levels. For instances, the g.7637G > A variant was reported related in increased risk of non-alcoholic fatty liver disease (Wang, Wang, Tong, Chang, & Wang, 2015) in China; and the variant of rs3737787 contributes to TG levels based on gender significant in Dutch FCHL families (J. C. Lee *et al*., 2007). Polymorphisms of upstream stimulatory factor 1 (USF1 306G > A) genes was found to be associated with plasma homocysteine level and susceptibility to ischemic stroke in a Korean population. (J. H. Lee *et al*., 2008). Latest study shows that, USF1 was identified as a novel factor regulating HDL functionality. The data from the study shows that USF1 inactivation boosts cholesterol efflux, attenuates macrophage cholesterol accumulation, linking improved macrophage cholesterol metabolism and inflammatory pathways to the antiatherogenic function of USF1 deficiency (Ruuth *et al*., 2018). However, USF1 could also be involved in coordinating the regulation of lipid and glucose metabolism by a mechanism not yet understood.

In the current study, genetic screening on normal individual in the Iban ethnic group in Sarawak, which is the largest state in Malaysia was conducted. This study aimed to analyse the association of single nucleotide polymorphisms (SNPs) of *USF 1* rs3737787 and lipid profile and LDL level. Genotype frequencies of this SNPs showed statistically significant difference for LDL level. The homozygous and homozygous genotypes of *USF 1* rs3737787 showed statistically significant association with LDL level. This result shows that in the Iban population, changes in *USF 1* rs3737787 associated with decreased of LDL level.

Single nucleotide polymorphisms (SNPs) with higher allele or genotype frequency from group of affected individuals are said to be at high risk with the specific disease (Lewis & Knight, 2012). Association study is the most applicable tool to access the gene susceptibility of complex diseases that involve high interaction between genetic and environmental factors. Many complex diseases have variety of genetic variants that affect the disease risk even though with minimal effect.

Genetic screening is considered a cost-effective strategy for detecting index cases of FH (Nordestgaard *et al*., 2013). It is necessary to identify FH susceptible allele in a population and screen the population for early and effective disease management. Very few population genetic studies have been reported from the Asian countries, though the Asian countries would have a different spectrum of mutations from Western countries. It is a challenging situation to conduct suitable genetic testing in Malaysia especially for the indigenous population in Sarawak. The number of Iban and Bidayuh individuals with Cardio vascular disease (CVD) was higher compared to other ethnic groups in Borneo (Sabah and Sarawak, Malaysia) (Fong *et al*., 2014).

Insufficient genetic testing is being carried out on a larger scale which can estimate the growing number of true FH patients. Current clinical diagnostic assessment without suitable genetic screening is unable to detect younger patients with FH as they will show no clear symptoms. FH is caused by several mutations found in autosomal dominant hypercholesterolemia (ADH) genes such *LDLR*, *APOB*, *PCSK9* and others (Marais, 2004).

CONCLUSION

In conclusion, to the best of our knowledge this is the first study of association of *USF 1* rs3737787 polymorphism and clinical data such as total cholesterol, LDL, HDL, Triglycerides and homocysteine level in the Iban ethnic group in the Malaysian population. Our results show that genetic diversity of *USF 1* rs3737787 influences the susceptibility to decreased level of LDL and support the involvement of *USF 1* mediated pathways in the process of FH.

ACKNOWLEDGEMENTS

We would like to express our gratitude towards Mr. Christerlikson ak Mathew, Ms. Mazuin and Gribble Laboratory staff who assisted us in subjects enrollment and blood sample collection., and continued to support towards this project. We also thank Universiti Malaysia Sarawak (UNIMAS) for the infrastructure provided. This work was supported by a grant from the Ministry of Higher Education, Malaysia with grant no. FRGS/2/2013/SKK01/UNIMAS/03/1.

REFERENCES

Corre, Sébastien, & Galibert, Marie□Dominique. (2005). Upstream stimulating factors: highly versatile stress□responsive transcription factors. *Pigment cell research*, 18(5), 337-348.

Fong, AYY, Zoriah, A, Omar, I, Amin, AN, Lim, CTY, & Feisul Idzwan, M. (2014). USE OF DRUGS FOR CARDIOVASCULAR DISORDERS. *MALAYSIAN STATISTICS ON MEDICINES 2009 & 2010*, 50.

Huijgen, R., Kindt, I., Defesche, J. C., & Kastelein, J. J. (2012). Cardiovascular risk in relation to functionality of sequence variants in the gene coding for the low-density lipoprotein receptor: a study among 29,365 individuals tested for 64 specific low-density lipoprotein-receptor sequence variants. *Eur Heart J*, 33(18), 2325-2330. doi: 10.1093/eurheartj/ehs038

Khoo, K. L., Van Acker, P., Tan, H., & Deslypere, J. P. (2000). Genetic causes of familial hypercholesterolaemia in a Malaysian population. *Med J Malaysia*, 55(4), 409-418.

Lee, Jae Ho, Kim, Ok Joon, Kim, Hyun Sook, Oh, Seung Hun, Jeung, Mingull, Kwon, Young Do, & Kim, Nam Keun. (2008). Polymorphisms of thymidylate synthase enhancer region (TSER) and upstream stimulatory factor 1 (USF1 306G→ A) genes are associated with plasma homocysteine level and susceptibility to ischemic stroke in a Korean population. *Genes Genom*, 30, 563-570.

Lee, Jenny C, Weissglas-Volkov, Daphna, Kyttälä, Mira, Sinsheimer, Janet S, Jokiaho, Anne, de Bruin, Tjerk WA, . . . van der Kallen, Carla JH. (2007). USF1 contributes to high serum lipid levels in Dutch FCHL families and US whites with coronary artery disease. *Arteriosclerosis, thrombosis, and vascular biology*, 27(10), 2222-2227.

Lewis, C. M., & Knight, J. (2012). Introduction to genetic association studies. Cold Spring Harb Protoc, 2012(3), 297-306. doi: 10.1101/pdb.top068163

Marais, A. David. (2004). Familial Hypercholesterolaemia. *The Clinical Biochemist Reviews*, 25(1), 49-68.

Meng, Li, Ruixing, Yin, Yiyang, Li, Xingjiang, Long, Kela, Li, Wanying, Liu, . . . Shangling, Pan. (2010). Association of LIPC-250G > A polymorphism and several environmental factors with serum lipid levels in the Guangxi Bai Ku Yao and Han populations. *Lipids in health and disease*, 9(1), 28.

Nordestgaard, B. G., Chapman, M. J., Humphries, S. E., Ginsberg, H. N., Masana, L., Descamps, O. S., . . . Tybjaerg-Hansen, A. (2013). Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J*, 34(45), 3478-3490a. doi: 10.1093/eurheartj/eht273

Pajukanta, Pälvi, Lilja, Heidi E, Sinsheimer, Janet S, Cantor, Rita M, Lusis, Aldons J, Gentile, Massimiliano, . . . Saarela, Janna. (2004). Familial combined hyperlipidemia is associated with upstream transcription factor 1 (USF1). *Nature genetics*, 36(4), 371.

Rajih, Alyaa, & Al-Talib, Hassanain. (2016). *Genetic researches among Malaysian familial hypercholesterolaemic population* (Vol. 201619).

Ruuth, Maija, Soronen, Jarkko, Kaiharju, Essi, Merikanto, Krista, Perttilä Julia, Metso, Jari, . . . Öörni, Katariina. (2018). USF1 deficiency alleviates inflammation, enhances cholesterol efflux and prevents cholesterol accumulation in macrophages. *Lipids in health and disease*, 17(1), 285.

Scriver, Charles R. (2001). *The metabolic & molecular bases of inherited disease* (Vol. 4): New York; Montreal: McGraw-Hill.

Shivraj, Livy, & Lye, Sayhean. (2011). *Familial Hypercholesterolemia in Asia: A Review* (Vol. 1).

Vasudevan, Ramachandran, Fathihah, Nurul, & Patimah, I. (2011). *Analysis of three polymorphisms in Bidayuh ethnic of Sarawak population: A report from Malaysia* (Vol. 10).

Wang, Ying, Wang, Bai-Fang, Tong, Jing, Chang, Bing, & Wang, Bing-Yuan. (2015). USF-1 genetic polymorphisms confer a high risk of nonalcoholic fatty liver disease in Chinese population. *International journal of clinical and experimental medicine*, 8(2), 2545.

Yamanaka, Tomoyuki, Tosaki, Asako, Kurosawa, Masaru, Shimogori, Tomomi, Hattori, Nobutaka, & Nukina, Nobuyuki. (2016). Genome□wide analyses in neuronal cells reveal that upstream transcription factors regulate lysosomal gene expression. *The FEBS journal*, 283(6), 1077-1087.