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ORIGINAL ARTICLE

No Association of Upstream Transcription Factor 1 gene (*USF1*) 306 G>A with Homocysteine level among Bidayuh Ethnic Groups in Sarawak Population

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

[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

*Corresponding Author: mmaminudin@unimas.my

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Abstract

This study was conducted to determine the polymorphic allele and genotype frequencies of *USF1* 306 G>A with homocysteine level and lipid profiles in Bidayuh ethnic group. It aimed to elucidate the association of the polymorphic allele and genotypes with lipid profiles such as total cholesterol (TC), high- density lipoprotein (HDL), low- density lipoprotein (LDL) and homocysteine level in Bidayuh ethnic group in Sarawak. One hundred and forty (140) individuals of the Bidayuh 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. Genotype frequency and allele frequencies of Upstream Transcription Factor 1 (*USF 1*) 306 G>A not associated with homocysteine level and other lipid profile among Bidayuh ethnic group. Our results show that the genetic diversity of *USF1* gene and allele does not influence the susceptibility of homocysteine level among Bidayuh ethnic group of the Malaysian population.

Keywords: Upstream Transcription Factor 1, Homocysteine, Bidayuh, Sarawak

Introduction

Homocysteine is an intermediate product of amino acid methionine and cysteine. It is produced via demethylation of dietary methionine that is found abundantly in animal protein (Faeh, Chiolero, & Paccaud, 2006). Hyperhomocysteinemia is a medical condition whereby higher level of homocysteine (more than 15 micromolar per liter) is detected in the blood (Guo, Chi, Xing, & Wang, 2009). Hyperhomocysteinemia may be influenced by genetic mutation on enzymes that are involved in homocysteine metabolism. Prevalence of hyperhomocystienemia varies between population and is dependent on age, diet and genetic background. High Homocysteine level is

associated with severe coronary artery disease in patients with coronary heart disease (Li et al., 2020).

Genetic trait of autosomal diseases that causes elevated blood cholesterol, called familial hypercholesterolemia (FH), and clarify on the workings of “component receptors” for low density lipoprotein (LDL), which is a form of cholesterol in bloodstream. In Fahed et al. (2011) studies, several genes have been shown to be involved in FH and yet determining the implications of the different mutations and polygenic on the phenotype remain a hard task for diagnostic purposes. This clearly demonstrated that the pattern of inheritance of FH is not specific or limited in/to one pattern on the gene.

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). *USF1* gene is commonly linked with familial combined hyperlipidemia (FCHL) (Pajukanta *et al*., 2004a). It is same with the autosomal dominant inheritance of LDLR gene but with complex polygenic.

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 et al., 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* 306 G>A 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 Bidayuh 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 forty (140) individuals of the Bidayuh 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 Bidayuh without inter-mixed 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 140 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'- GTGGCCAGGCCCTCAGAA-3' (wildtype) or 5'- GTGGCCAGGCCCTCAGAG -3' (variant). The consensus primer used was 5'- GAGATGGAGTGAAGTTTGGA -3' (giving a PCR product of 124 bp). The *USF 1* 306 G>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* 306 G>A polymorphic genotype was categorized into homozygous wild, heterozygous and homozygous variant.

Statistical Analysis

All genotypes and allele frequencies were calculated. Genotype *USF 1* 306 G>A association with lipid profile and homocysteine level were calculated using One Way ANOVA and confirmed by post hoc analysis Tukey Test to determine the parameter involved. As for association of allele of *USF 1* 306 G>A with lipid profile and homocysteine level were calculated using Independent Sample T test.

Results and Discussion

Genotype frequencies of USF 1 306 G>A with Homocysteine level and Lipid Profiles

On comparing genotype frequencies of *USF1* 306 G>A with homocysteine level and lipid profile, all clinical data do not show any statistically significant when compared with genotypes of *USF1* 306 G>A as shown in Table 1.

When comparing levels of homocysteine with genotype frequencies of *USF1* 306 G>A, there are not statistically significant differences observed with p value of 0.21.

Table 1. Genotype frequencies of *USF1* 306 G>A and Clinical data

Clinical Data	***USF1 306* G>A**				
	Wildtype (GG)	**Heterozygous (GA)**	**Variant (AA)**	**Total**	**p value**
Total Cholesterol < 6.2 mmol/L	26	71	27	124	0.42

≥ 6.2 mmol/L	2	8	6	16	
LDL					
< 4.1 mmol/L	28	71	29	128	0.15
≥ 4.1 mmol/L	0	8	4	12	
HDL					
≥ 1 mmol/L	23	68	27	118	0.77
< 1 mmol/L	5	11	6	22	
Triglycerides					
< 2.3 mmol/L	26	61	23	110	0.06
≥ 2.3 mmol/L	2	18	10	30	
Homocysteine					
< 15 µmol/L	17	43	13	73	0.21
≥ 15 µmol/L	11	36	20	67	

Allele frequencies of USF 1 306 G>A with Homocysteine level and Lipid Profiles

Table 2 shows allele frequencies of *USF* 306 G>A and Clinical data. When comparing levels of homocysteine with allele frequencies of *USF1* 306 G>A, there are not statistically significant differences observed with p value of 0.11. On comparing allele frequencies of *USF1* 306 G>A with other lipid profiles also not show any statistically significant with p value more than 0.05.

Table 2. Allele frequencies of USF 306 G>A and Clinical data

Clinical Data	**USF 306 G>A**			
	A	**G**	**Total**	**p value**
Total Cholesterol				
< 6.2 mmol/L	117	118	235	0.24
≥ 6.2 mmol/L	17	26	43	
LDL				
< 4.1 mmol/L	132	138	270	0.28
≥ 4.1 mmol/L	2	6	8	
HDL				
≥ 1 mmol/L	113	121	234	0.15
< 1 mmol/L	23	39	62	
Triglycerides				
< 2.3 mmol/L	111	105	216	0.06
≥ 2.3 mmol/L	23	39	62	
Homocysteine				
< 15 µmol/L	77	69	146	0.11
≥ 15 µmol/L	57	75	132	

Association of genotype and allele frequencies of USF 1 306 G>A with Homocysteine level and Lipid Profiles.

Table 3 shows association of genotype frequencies of USF 306 G>A and all lipid profiles data. There are no significant associations observed in association of genotype frequencies of *USF 1* 306 G>A with homocysteine level with F (1.571) and p value of 0.211.

When confirmation analysis for the association of genotype frequencies of USF 306 G>A and Homocysteine level conducted, there are no statistical association observed between USF 306 G>A and Homocysteine level as shown in Table 4.

Association of allele frequencies of *USF 1* 306 G>A and clinical data also shows no significant associations observed in association of allele frequencies of *USF 1* 306 G>A with homocysteine level with t (-1.571) and p value of 0.112 (Table 5).

Table 3. Association genotype frequencies of USF 306 G>A and Clinical data

		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Total Cholesterol	Between Groups	.215	2	.108	1.057	.350
	Within Groups	13.956	137	.102		
	Total	14.171	139			
LDL	Between Groups	.266	2	.133	1.705	.186
	Within Groups	10.705	137	.078		
	Total	10.971	139			
HDL	Between Groups	.058	2	.029	.216	.806
	Within Groups	18.485	137	.135		
	Total	18.543	139			
Triglycerides	Between Groups	.846	2	.423	2.550	.082
	Within Groups	22.726	137	.166		
	Total	23.571	139			
Homocysteine	Between Groups	.783	2	.392	1.571	.211
	Within Groups	34.152	137	.249		
	Total	34.936	139			

Table 4. Tukey HSD test on *USF 306* G>A and Homocysteine

(I) LDLRARG	**(J) LDLRARG**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval Lower Bound**	**Upper Bound**
Wildtype	Hetero	-.063	.110	.835	-.32	.20
	Variant	-.213	.128	.224	-.52	.09
Hetero	Wildtype	.063	.110	.835	-.20	.32
	Variant	-.150	.103	.317	-.40	.09
Variant	Wildtype	.213	.128	.224	-.09	.52
	Hetero	.150	.103	.317	-.09	.40

Table 5. Association of allele frequencies of *USF 1* 306 G>A and clinical data

		t	df	Sig. (2-tailed)	95% Confidence Interval of the Difference Lower	Upper
Total Cholesterol	Equal variances assumed	-1.236	276	.218	-.054	.043
	Equal variances not assumed	-1.242	274.582	.215	-.054	.043
LDL	Equal variances assumed	-1.332	276	.184	-.027	.020
	Equal variances not assumed	-1.354	238.454	.177	-.027	.020
HDL	Equal variances assumed	-.068	276	.946	-.003	.044
	Equal variances not assumed	-.068	274.846	.946	-.003	.044
Triglycerides	Equal variances assumed	-1.992	276	.047	-.099	.050
	Equal variances not assumed	-2.004	273.725	.046	-.099	.049
Homocyctiene	Equal variances assumed	-1.594	276	.112	-.095	.060
	Equal variances not assumed	-1.595	274.939	.112	-.095	.060

Study showed that increased homocysteine levels are associated with high cardio vascular disease (CVD) risk among middle-aged and elderly populations in Taiwan (Shih et al., 2021). Another study showed an elevated homocysteine level was a risk factor for CVD (Chrysant & Chrysant, 2018). It has been believed that homocysteine disrupts endothelial function, leading to vessel damage and, ultimately, to CVD (Balint et al., 2020; Finch & Joseph, 2010). In the current study, genetic screening on normal individual in the Bidayuh 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* 306 G>A and lipid profile. Both the allele and genotype frequencies of this SNPs not showed statistically significant in all analysis. The association analysis also not show significant association of *USF 1* 306 G>A with all lipid profile tested.

Current study in contrast with study in Iban ethnic group, genotype frequency of *USF 1* 306 G>A showed statistical significant difference with homocysteine level with p value less than 0.05 (Mustapha et al., 2020). In the same study also, heterozygous, and variant genotypes of *USF 1* 306 G>A was found to be significantly associated with high level of homocysteine with $F(2,112) = 7.048$, $p < 0.05$. The variant allele of USF 1 306 G > A is also significantly associated with high level of triglycerides with t (-2.116), p value of 0.035. Thus, the result showed that the genetic diversity of *USF1* gene influences the susceptibility to increased level of homocysteine in the Iban ethnic group of the Malaysian population. Latest study of polymorphisms of *USF 1* gene, *USF1* rs3737787 showed influences the susceptibility to decreased level of LDL in the Iban ethnic group of the Malaysian population (Mustapha et al., 2021).

Study on Bidayuh ethnic group in Sarawak population showed that the genetic diversity of *PCSK9* gene influences the susceptibility to increased level of homocysteine (Mustapha et al., 2019b). The PCSK9 protein controls the number of low-density lipoprotein receptors, which are

proteins on the surface of cells. These receptors play a critical role in regulating blood cholesterol levels. Another study show that, showed that the genetic diversity of *LDLR* gene influences the susceptibility to increased level of homocysteine in Iban ethnic group in in Malaysian population (Mustapha et al., 2019a). The LDLR gene provides instructions for making a protein called the low-density lipoprotein receptor. This receptor binds to particles called low-density lipoproteins (LDLs), which are the primary carriers of cholesterol in the blood.

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 populations 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) (Fong et al., 2014).

Conclusion

In conclusion, our results show that the genetic diversity of *USF1 USF 1* 306 G>A gene and allele does not influence the susceptibility to increase or decrease level of homocysteine in the Bidayuh ethnic group of the Malaysian population.

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References

Balint, Brittany, Jepchumba, Viola Kosgei, Guéant, Jean-Louis, & Guéant-Rodriguez, Rosa-Maria. (2020). Mechanisms of homocysteine-induced damage to the endothelial, medial and adventitial layers of the arterial wall. *Biochimie, 173*, 100-106.

Chrysant, Steven G, & Chrysant, George S. (2018). The current status of homocysteine as a risk factor for cardiovascular disease: a mini review. *Expert review of cardiovascular therapy, 16*(8), 559-565.

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

Faeh, D., Chiolero, A., & Paccaud, F. (2006). Homocysteine as a risk factor for cardiovascular disease: should we (still) worry about? *Swiss Med Wkly, 136*(47-48), 745-756. doi: 2006/47/smw-11283

Fahed, Akl C, Safa, Raya M, Haddad, Fadi F, Bitar, Fadi F, Andary, Rabih R, Arabi, Mariam T, . . . Nemer, Georges. (2011). Homozygous familial hypercholesterolemia in Lebanon: a genotype/phenotype correlation. *Molecular genetics and metabolism, 102*(2), 181-188.

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.

Guo, H., Chi, J., Xing, Y., & Wang, P. (2009). Influence of folic acid on plasma homocysteine levels & arterial endothelial function in patients with unstable angina. *Indian J Med Res, 129*(3), 279-284.

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

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

Li, Shanshan, Sun, Liping, Qi, Liqun, Jia, Yongsheng, Cui, Zhuo, Wang, Zhongsuo, . . . Zhao, Xiaofeng. (2020). Effect of high homocysteine level on the severity of coronary heart disease and prognosis after stent implantation. *Journal of cardiovascular pharmacology, 76*(1), 101-105.

M Finch, Jennifer, & Joseph, Jacob. (2010). Homocysteine, cardiovascular inflammation, and myocardial remodeling. *Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders), 10*(4), 241-245.

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.

Mustapha, Mohd Aminudin, Sim, Sai-Peng, Hood, Hafizah Hanis, & Ted, Siaw Yun. (2020). Association of Upstream Transcription Factor 1 Gene (USF1) 306 G> A with Increased Homocysteine Level among Iban Ethnic Groups in Sarawak Population. *International Medical Journal, 27*(3), 273-276.

Mustapha, Mohd Aminudin, Sim, Sai-Peng, Hood, Hafizah Hanis, & Ted, Siaw Yun. (2021). Association of Upstream Transcription Factor 1 Gene (USFl) rs3737787 with Reduced LDL (low-density lipoprotein) Cholesterol Level among Iban Ethnic Groups in Sarawak Population. *International Medical Journal, 28*(2).

Mustapha, Mohd Aminudin, Sim, Sai-Peng, Hood, Hafizah Hanis, Ted, Siaw Yun, Ling, Maybelline Goh Boon, & Hazmi, Helmy. (2019a). Association of Low-Density Lipoprotein Receptor (LDLR) Arg471Gly with Increased Homocysteine Level among the Iban Ethnic Group in the Sarawak Population. *International Medical Journal, 26*(4), 270-274.

Mustapha, Mohd Aminudin, Sim, Sai-Peng, Hood, Hafizah Hanis, Ted, Siaw Yun, Ling, Maybelline Goh Boon, & Hazmi, Helmy. (2019b). Association of PCSK9 g. 24382G> A with Increased Homocysteine Level among Bidayuh Ethnic Group in Sarawak Population. *International Medical Journal, 26*(3), 172-176.

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, Päivi, 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.

Shih, Chin-Chuan, Shih, Yu-Lin, & Chen, Jau-Yuan. (2021). The association between homocysteine levels and cardiovascular disease risk among middle-aged and elderly adults in Taiwan. *BMC Cardiovascular Disorders, 21*(1), 1-8.

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

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.

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