ORIGINAL ARTICLE

No Association between Upstream Transcription Factor 1 Gene (*USF1*) 306 G > A Variant and Homocysteine Levels among Bidayuh Ethnic Groups in the Sarawak Population

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

ABSTRACT

Objective: **This study aimed to determine the allele and genotype frequencies of the *USF1* 306 G > A polymorphism and its association with homocysteine levels and lipid profiles in the Bidayuh ethnic group.**

Material and Methods: **A total of 140 individuals from the Bidayuh ethnic group participated in this study. Genotyping was performed using Allele-Specific Polymerase Chain Reaction (AS-PCR). The association between genotype frequencies and clinical profiles was assessed using One-Way ANOVA, while Independent Sample T-tests were employed to analyze the association between allele frequencies and clinical profiles.**

Results: **Our findings revealed that genotype and allele frequencies of the *USF1* 306 G > A polymorphism were not associated with homocysteine levels among the Bidayuh ethnic group.**

Conclusion: **Therefore, our results suggest that the genetic diversity of the *USF1* gene and its alleles do not influence susceptibility to elevated homocysteine levels in the Bidayuh ethnic group of the Malaysian population.**

KEY WORDS

upstream transcription factor 1, homocysteine, Bidayuh, Sarawak

INTRODUCTION

Homocysteine is an intermediate product of the amino acids methionine and cysteine. It is produced via the demethylation of dietary methionine, which is abundant in animal protein[1)]. Hyperhomocysteinemia is a medical condition characterized by elevated levels of homocysteine in the blood, typically exceeding 15 micromoles per liter[2)]. Genetic mutations in enzymes involved in homocysteine metabolism can contribute to hyperhomocysteinemia. The prevalence of hyperhomocysteinemia varies among populations and depends on factors such as age, diet, and genetic background. Elevated homocysteine levels are associated with severe coronary artery disease in patients with coronary heart disease[3)].

The upstream transcription factors 1 (*USF1*) and *USF2* belong to the basic helix-loop-helix/leucine zipper transcription factor family[4)]. Moreover, USFs have been demonstrated to regulate the expression of genes involved in fatty acid synthesis and insulin signaling, suggesting their role in glucose and lipid metabolism[5)]. T The *USF1* gene is situated on chromosome 1q22-q23, spanning 11 exons and extending over 6.73 kb. Genetic association studies have linked USF1 to Coronary Artery Disease (CAD) in Finnish families[6)]. Additionally, *USF1* has been implicated in hypercholesterolemia and shown to predispose individuals to premature cardiovascular diseases[7)]. The *USF1* gene is commonly associated with familial combined hyperlipidemia (FCHL)[6)], exhibiting similar traits to the autosomal dominant inheritance pattern of the LDLR gene but with a complex polygenic nature..

Sarawak, the largest state in Malaysia, has a population of 2.6 million people, with indigenous groups comprising approximately 50% of the total population. The Iban ethnic group is the largest, accounting for 38% of the population, followed by the Bidayuh, making up about 10%[8)]. Studies have indicated that the incidence of Coronary Vascular Disease (CVD) among the Iban and Bidayuh ethnic groups in Borneo (Sabah & Sarawak) is higher compared to other ethnic groups[9)].

Untreated Familial hypercholesterolemia (FH) patients have a 3-4 times higher risk of developing coronary heart disease compared to individuals without FH[10)]. Although there is a relationship between the *USF1* gene and FH, genetic data are inadequate in the Malaysian population, and none have been reported in Sarawak. Therefore, the current study was conducted to determine the polymorphic allele and genotype frequencies of *USF1* 306 G > A and to elucidate the association of these polymorphisms with homocysteine levels in the Bidayuh ethnic group in Sarawak.

MATERIALS AND METHODS

OR
Participant Recruitment

The study commenced after obtaining approval from the Research Review Board and Ethics Committee of Universiti Malaysia Sarawak (UNIMAS). All subjects provided informed consent before participating

Received on April 1, 2024 and accepted on April 27, 2024

ORCHID ID:
Mohd Aminudin Mustapha: 0000-0002-7457-554X

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
(email: mmaminudin@unimas.my)

Table 1: Genotype frequencies of *USF1* 306 G > A and Homocysteine

Clinical Data	*USF1* 306 G > A				
	Wildtype (GG)	Heterozygous (GA)	Variant (AA)	Total	p value
Homocysteine					
< 15 µmol/L	17	43	13	73	0.21
≥ 15 µmol/L	11	36	20	67	

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

		Sum of Squares	df	Mean Square	F	Sig.
Homocysteine	Between Groups	.783	2	.392	1.571	2ll
	Within Groups	34.152	137	.249		
	Total	34.936	139			

in the research. One hundred and forty (140) individuals from the Bidayuh ethnic group in Sarawak were enrolled as study subjects. Each participant was required to fast for 10 hours before blood sampling, and participants had to be over 18 years of age.

Inclusion criteria

The inclusion criteria were based on individuals of Bidayuh ethnicity who had not participated in intermarriage with other ethnic groups for up to two generations.

Exclusion criteria

Respondents currently under medication for hypertension or hypercholesterolemia, as well as those who underwent major surgery within 6 months prior to the study, were excluded.

DNA extraction

Peripheral blood samples were collected from 140 individuals of the Iban ethnic group in EDTA tubes after obtaining written informed consent. The samples were stored at -20°C. Genomic DNA extraction was performed using the QIAamp DNA Blood Mini Kit (QIAGEN), followed by amplification of the gene of interest using appropriate primers.

Genotyping

The allele-specific primers used were 5'-GTGGCCAGGCCCTCAGAA-3' for the wildtype allele and 5'-GTGGCCAGGCCCTCAGAG-3' for the variant allele. A consensus primer, 5'-GAGATGGAGTGAAGTTTGGA-3', was employed to produce a PCR product of 124 bp. The USF1 306 G > A SNP was analyzed using allele-specific PCR. PCR reactions were performed using a master mix containing 80 ng DNA template, primers (0.2 uM each), 2.0 mM MgCl2, 10x buffer, 10 mM dNTPs (0.2 mM each), and 1.25 units of Taq DNA polymerase (GoTaq® Flexi DNA Polymerase) in a total volume of 25 µl. The PCR products were separated on a 2% agarose gel and visualized using ethidium bromide staining. The *USF1* 306 G > A polymorphic genotypes were categorized as homozygous wildtype, heterozygous, and homozygous variant.

Statistical Analysis

All genotype and allele frequencies were calculated. The association between the *USF1* 306 G > A genotype and homocysteine levels was assessed using one-way ANOVA, confirmed by post-hoc analysis using the Tukey test to determine the involved parameters. The association of the *USF1* 306 G > A allele with homocysteine levels was analyzed using independent sample t-tests.

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

Clinical Data	USF 306 G > A			
	A	G	Total	p value
Homocysteine				
< 15 µmol/L	77	69	146	0.11
≥ 15 µmol/L	57	75	132	

RESULTS

Genotype Frequencies of *USF1* 306 G > A in Relation to Homocysteine Levels.

The comparison of genotype frequencies of *USF1* 306 G > A with homocysteine levels did not reveal any statistically significant differences, as shown in Table 1. Similarly, when comparing homocysteine levels with genotype frequencies of *USF1* 306 G > A, no statistically significant differences were observed, with a p-value of 0.21.

Allele frequencies of *USF1* 306 G > A with Homocysteine level.

Table 2 displays the allele frequencies of *USF1* 306 G > A and homocysteine levels. When comparing homocysteine levels with allele frequencies of *USF1* 306 G > A, no statistically significant differences were observed, with a p-value of 0.11.

Association of genotype and allele frequencies of *USF1* 306 G>A with Homocysteine level.

Table 3 presents the association between genotype frequencies of *USF1* 306 G > A and homocysteine levels. No significant associations were observed, with an F value of 1.571 and a p-value of 0.211.

Confirmation analysis for the association between genotype frequencies of *USF1* 306 G > A and homocysteine levels was conducted, revealing no statistical association between *USF1* 306 G > A and homocysteine levels, as depicted in Table 4.

The association between allele frequencies of *USF1* 306 G > A and homocysteine levels revealed no significant associations, with a t-value of -1.571 and a p-value of 0.112 (see Table 5).

DISCUSSION

Studies have indicated that elevated homocysteine levels are associated with an increased risk of cardiovascular disease (CVD) among middle-aged and elderly populations in Taiwan[11], as well as being recognized as a risk factor for CVD in other research[12]. Homocysteine is believed to disrupt endothelial function, leading to vessel damage and ultimately contributing to CVD development[13,14]. In this study, genetic screening was conducted on normal individuals from the Bidayuh ethnic group in Sarawak, the largest state in Malaysia. The aim was to analyze the association between single nucleotide polymorphisms (SNPs) of *USF1* 306 G > A and homocysteine level.

In the current study, both the allele and genotype frequencies of *USF1* 306 G > A did not show statistically significant associations with homocysteine levels. Additionally, the association analysis did not reveal a significant association between *USF1* 306 G > A and homocysteine levels in the Bidayuh ethnic group. These results suggest that genetic diversity in the *USF1* 306 G > A does not influence susceptibility to increased levels of homocysteine in the Bidayuh ethnic group of the Malaysian population. In contrast, in the Iban ethnic group study[15], the study revealed a statistically significant difference in the genotype frequency of *USF1* 306 G > A with homocysteine levels. Furthermore, in the same study, heterozygous and variant genotypes of *USF1* 306 G > A were significantly associated with high levels of homocysteine. Another study on polymorphisms of the *USF1* gene, specifically *USF1* rs3737787, revealed an influence on susceptibility to decreased levels of LDL in the Iban ethnic group[16]. A similar study conducted on the Bidayuh ethnic group in the Sarawak population revealed that genetic diversity in the *PCSK9* gene influences susceptibility to increased levels

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	95% Confidence Interval 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	95% Confidence Interval of the Difference Upper
Homocyctiene	Equal variances assumed	-1.594	276	.112	-.095	.060
	Equal variances not assumed	-1.595	274.939	.112	-.095	.060

of homocysteine[17]. Genetic diversity in the LDLR gene was found to influence susceptibility to increased levels of homocysteine in the Iban ethnic group[18].

Single nucleotide polymorphisms (SNPs) with higher allele or genotype frequencies among affected individuals are considered to be at higher risk for specific diseases[19]. Association studies are the most applicable tools for assessing gene susceptibility in complex diseases involving interactions between genetic and environmental factors. Many complex diseases are influenced by a variety of genetic variants, even those with minimal effects. Genetic screening is considered a cost-effective strategy for detecting index cases of familial hypercholesterolemia (FH)[20]. It is crucial to identify FH-susceptible alleles in a population and screen individuals for early and effective disease management. Despite the unique genetic spectrum in Asian countries, few population genetic studies have been reported. Conducting suitable genetic testing in Malaysia, especially for indigenous populations in Sarawak, presents challenges. Additionally, the number of individuals from the Iban and Bidayuh ethnic groups with cardiovascular disease (CVD) is higher compared to other ethnic groups in Borneo (Sabah and Sarawak, Malaysia)[9].

CONCLUSION

In conclusion, our results indicate that the genetic diversity of the *USF1* gene, specifically the *USF1* 306 G > A allele, does not influence the susceptibility to increased or decreased levels of homocysteine in the Bidayuh ethnic group of the Malaysian population.

ACKNOWLEDGEMENTS

We express our gratitude to Mr. Christerlikson ak Mathew, Ms. Mazuin, and the Gribble Laboratory staff for their assistance in subject enrollment, blood sample collection, and ongoing support for this project. We also extend our thanks to Universiti Malaysia Sarawak (UNIMAS) for providing the necessary infrastructure. 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

1. Faeh D, Chiolero A, Paccaud F. Homocysteine as a risk factor for cardiovascular disease: should we (still) worry about? Swiss Med Wkly. 2006 Dec 2; 136(47-48): 745-56. PubMed PMID: 17225194. Epub 2007/01/17. eng.
2. Guo H, Chi J, Xing Y, Wang P. Influence of folic acid on plasma homocysteine levels & arterial endothelial function in patients with unstable angina. Indian J Med Res. 2009 Mar; 129(3): 279-84. PubMed PMID: 19491420. Epub 2009/06/06. eng.
3. Li S, Sun L, Qi L, Jia Y, Cui Z, Wang Z, *et al*. Effect of high homocysteine level on the severity of coronary heart disease and prognosis after stent implantation. Journal of cardiovascular pharmacology. 2020; 76(1): 101-5.
4. Yamanaka T, Tosaki A, Kurosawa M, Shimogori T, Hattori N, Nukina N. Genome?wide analyses in neuronal cells reveal that upstream transcription factors regulate lysosomal gene expression. The FEBS journal. 2016; 283(6): 1077-87.
5. Corre S, Galibert MD. Upstream stimulating factors: highly versatile stress?responsive transcription factors. Pigment cell research. 2005; 18(5): 337-48.
6. Pajukanta P, Lilja HE, Sinsheimer JS, Cantor RM, Lusis AJ, Gentile M, *et al*. Familial combined hyperlipidemia is associated with upstream transcription factor 1 (USF1). Nature genetics. 2004; 36(4): 371-6.
7. Meng L, Ruixing Y, Yiyang L, Xingjiang L, Kela L, Wanying L, *et al*. 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. 2010; 9(1): 28.
8. Vasudevan R, Fathihah N, Patimah I. Analysis of three polymorphisms in Bidayuh ethnic of Sarawak population: A report from Malaysia2011. 4544-9 p.
9. Fong A, Zoriah A, Omar I, Amin A, Lim C, Feisul Idzwan M. USE OF DRUGS FOR CARDIOVASCULAR DISORDERS. MALAYSIAN STATISTICS ON MEDICINES 2009 & 2010. 2014: 50.
10. Huijgen R, Kindt I, Defesche JC, Kastelein JJ. 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. 2012 Sep; 33(18): 2325-30. PubMed PMID: 22390909. Epub 2012/03/07. eng.
11. Shih C-C, Shih Y-L, Chen J-Y. The association between homocysteine levels and cardiovascular disease risk among middle-aged and elderly adults in Taiwan. BMC Cardiovascular Disorders. 2021; 21(1): 1-8.
12. Chrysant SG, Chrysant GS. The current status of homocysteine as a risk factor for cardiovascular disease: a mini review. Expert review of cardiovascular therapy. 2018; 16(8): 559-65.
13. Balint B, Jepchumba VK, Guéant J-L, Guéant-Rodriguez R-M. Mechanisms of homocysteine-induced damage to the endothelial, medial and adventitial layers of the arterial wall. Biochimie. 2020; 173: 100-6.
14. M Finch J, Joseph J. Homocysteine, cardiovascular inflammation, and myocardial remodeling. Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders). 2010; 10(4): 241-5.
15. Mustapha MA, Sim S-P, Hood HH, Ted SY. 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. 2020; 27(3): 273-6.
16. Mustapha MA, Sim S-P, Hood HH, Ted SY. 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. 2021; 28(2).
17. Mustapha MA, Sim S-P, Hood HH, Ted SY, Ling MGB, Hazmi H. Association of PCSK9 g. 24382G > A with Increased Homocysteine Level among Bidayuh Ethnic Group in Sarawak Population. International Medical Journal. 2019; 26(3): 172-6.
18. Mustapha MA, Sim S-P, Hood HH, Ted SY, Ling MGB, Hazmi H. Association of Low-Density Lipoprotein Receptor (LDLR) Arg471Gly with Increased Homocysteine Level among the Iban Ethnic Group in the Sarawak Population. International Medical Journal. 2019; 26(4): 270-4.
19. Lewis CM, Knight J. Introduction to genetic association studies. Cold Spring Harbor protocols. 2012 Mar 1; 2012(3): 297-306. PubMed PMID: 22383645. Epub 2012/03/03. eng.
20. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. 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. European heart journal. 2013 Dec; 34(45): 3478-90a. PubMed PMID: 23956253. Pubmed Central PMCID: PMC3844152. Epub 2013/08/21. eng.