International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Analysis of Sex and ABO Blood Group on Vascular Endothelial Growth Factor-A and Apolipoprotein-E Gene Polymorphisms in First Descendant of Coronary Heart Disease Patients

Chariza Mauli Ananda Pane[1], Rahmawaddah Yolanda[1], Aurora Fajria A[1], Elvira Yunita[2], Novriantika Lestari[3], Ismir Fahri[4], Ellen Maidia Djatmiko[2], Marisadonna Asteria[5], Qomariah Hasanah[6]*

[1]*Study Program of Medicine, Faculty of Medicine and Health Sciences Universitas Bengkulu, Indonesia.*
[2]*Department of Biochemistry and Molecular Biology, Faculty of Medicine and Health Sciences Universitas Bengkulu, Indonesia.*
[3]*Department of Pharmacology, Faculty of Medicine and Health Sciences Universitas Bengkulu, Indonesia.*
[4]*Department of Cardiology and Vascular Medicine, RSUD M.Yunus Bengkulu, Indonesia.*
[5]*Department of Anatomical Pathology, Faculty of Medicine and Health Sciences Universitas Bengkulu, Indonesia.*
[6]*Study Program in Natural Sciences, Faculty of Tarbiyah dan Tadris, UIN Fatmawati Sukarno Bengkulu, Indonesia.*

Abstract

Apolipoprotein E (Apo-E) gene polymorphism with the E4 allele has a higher risk of atherosclerosis that develops into coronary heart disease (CHD). Vascular Endothelial Growth Factor a (VEGF-A) gene polymorphisms can indicate the severity of coronary lesions, and play a role in the development of CHD. Some of the risk factors for CHD include gender and blood type. This study aims to analyse the prevalence of sex and blood type of Apo-E and VEGF-A gene polymorphisms in Bengkulu City. This research is descriptive observational research with a cross-sectional study design. The variables in this research were the respondent's gender and blood group which are analysed for their relationship with Apo-E and VEGF-A gene polymorphisms. The relationship between variables was analysed using the Chi-Square Test. The results of this study showed that there was a significant relationship between gender and Apo-E gene polymorphisms (p: 0.009) and VEGF-A (p: 0.008) in the first descendant of CHD. Odds ratio analysis showed that men were 17.5 times more at risk of having Apo-E4 gene polymorphisms and 24 times more at risk of having VEGF-A gene polymorphisms with CC genotype in the first generation of patients with CHD. Blood type was also associated with Apo-E (p: 0.002) and VEGF-A (p: 0.024) gene polymorphisms in the first generation of CHD patients. Odds ratio analysis showed that the first generation of CHD with non-O blood type had a 38.5 times risk of having Apo-E4 gene polymorphisms and 15.75 times the risk of having VEGF-A gene polymorphisms with the CC genotype. Male sex and non-O blood type in the first derivatives of CHD are more at risk of having Apo-E and VEGF-A gene polymorphisms which are more susceptible to CHD events.

Keywords: Apo-E, blood group, CHD, sex, VEGF-A

Full length article **Corresponding Author*, e-mail: elvirayunita@unib.ac.id

1. Introduction

The prevalence of CHD in Indonesia is around 883,447 people. Meanwhile, Bengkulu province is estimated to have 3,748 people, or around 1.3% [1]. The incidence of CHD occurs is influenced by several risk factors. The CHD risk factors are modifiable and non-modifiable risk factors. Smoking, hypertension, diabetes, and alcohol consumption are modifiable factors, while the genetics, gender, ABO blood group, and age are non-modifiable factors. In genetic aspect, Single nucleotide polymorphisms (SNPs) also play a crucial role in the development of CHD [2,3]. The sex factor with the incidence of gene polymorphisms in CHD has a fairly close relationship [4]. In addition to gender, the ABO blood group also has a risk in the development of CHD. However, there is still lack of data that studies examining the effect of blood type on gene polymorphisms in the first derivative patients with CHD, especially in Bengkulu City. Apo-E and VEGF gene polymorphisms play a critical role in the development of atherosclerosis. The three isoforms of Apo-E are ε2 (Cys112, Cys158), ε3 (Cys112, Arg158), and ε4 (Arg112, Arg158) alleles. The ε3 allele usually is the major allele, while the ε2 and ε4 alleles are the variants. The ε4 allele at the genetic level has a higher risk of atherosclerosis [5–7]. VEGF is an important promoter of lymphomagenesis and angiogenesis. Previous studies reported that elevated plasma VEGF-A levels in CHD patients suggest the severity of coronary lesions, and can be considered as an important indicator of revascularization [8–10]. Identification of the Apo-E and VEGF-A gene polymorphisms with gender and

blood group was carried out to determine the effect of these two factors on the first derivative of patients with CHD. This study was conducted because no data are reporting on the prevalence of sex and blood type of Apo-E gene polymorphisms and VEGF-A gene polymorphisms in the first generation of CHD patients in Bengkulu City. The main objective of this study was to analyze the prevalence of sex and blood type of Apo-E and VEGF-A gene polymorphisms in the first descendant of patients with coronary heart disease in Bengkulu city. The results of this study are looked forward to be a reference for carrying out early preventive actions for the patient's family and the community to reduce the risk of CHD.

2. Materials and methods

This research is a cross-sectional study design. Retrieval of data used is primary data taken directly by filling out a questionnaire. The instruments in this research are the characteristics of the respondents such as gender and blood type. This research was conducted in the city of Bengkulu. Primary data management will be taken using a questionnaire in the period November 2021 - September 2022. The population in this study is the first derivatives of CHD sufferers in Bengkulu city. The research sample size was calculated based on the categorical descriptive formula (unpaired categorical analysis) [11] so that a sample size of 42 people was obtained. Genetic polymorphism data is taken as secondary data from our previous studies that have been reported [12,13]. The inclusion criteria carried out in this study were: 1) The first derivative of patients with CHD in Bengkulu City; and 2) Inclined to be a research subject and agree to sign informed consent. Exclusion criteria in this research were: 1) Subjects have a history of psychiatric disorders; 2) Refuse to be a research subject. The dropout criteria in this study were the research subjects withdrew from the study or did not complete the procedure. The research subject for first-descendant CHD patients was taken through medical records from RSUD dr. M. Yunus Bengkulu. The first derivative of non-CHD in this research was conducted by filling out the WHO Rose Angina questionnaire through online-based media. The data obtained will be analyzed using the Statistical Program for Social Science 7.0 application with the Chi-Square test.

3. Results and Discussions

3.1. Subject Characteristics

Table 1 shows the data obtained on the subject's parents who had a history of coronary heart disease (CHD) as many as 21 people (50.00%) and 21 people without CHD (50.00%). Meanwhile, overall research subjects found more women (66.70%) compared to men (33.30%). Most of the research subjects had blood type O (54.80%) compared to other blood types. This research involved 42 subjects who were divided into two categories, the first generation of patients with CHD and non-CHD patients. Subject characteristics consisted of sex, blood type, Apo-E gene polymorphism in the sample, and VEGF-A. Gender characteristics in this study were dominated by women compared to men. Several studies have shown that the incidence of CHD in men has a three times higher risk and five times higher mortality compared to women. The main differences in risk factors for sex are HDL cholesterol levels, body mass index, diabetes prevalence, and smoking habits. This can increase the risk of CHD higher in men compared to women [4,14]. Meanwhile, the results of subsequent studies showed that there were no significant gender differences from various risk factors, except that the risk of CHD continued to increase in people with smoking habits which are usually done by men. This is because smoking affects the atherogenic lipoprotein cholesterol profile in young adults. Gender differences between women and men in the level of risk factors will decrease with increasing age [15]. This study was also dominated by respondents with blood type O, B, and A. The previous study reported that non-O blood groups, blood types A, B, and AB had a higher risk of CHD events than blood type O which has a lowest risk of cardiovascular disease such as CHD [16]. In addition our previous study also showed that most of research subjects had the E3 genotype in Apo-E polymorphism and CC genotype in VEGF-A polymorphism.

3.2. Prevalence of Sex and Blood Types of Apo-E Gene Polymorphisms

The results revealed that there was a significant relationship between sex and Apo-E gene polymorphisms in the first d of CHD patients in Bengkulu City (p: 0.009). The history of non-CHD first offspring having the E2 and E3 alleles was also dominated by women (92.90%). This is different from the non-PJK first derivative. Besides that, there was no significant relationship between sex and Apo-E gene polymorphisms in the first non-CHD offspring in Bengkulu city (p: 1,000). The odds ratio value obtained for the first generation of CHD sufferers is 17.50 (95% CI 1.96-15.59), while in the first derivative of non-CHD it is 2.16 (95% CI 0.11-40.88). This indicates that first-born males with CHD have a 17.50 times higher risk of developing Apo-E4 polymorphisms than non-CHD first-born males (Table 2). Apo-E protein main role is as lipid transporters (fats and cholesterol) in the blood. Apo-E recognized by specific cell surface receptors that will carry lipids to cells for storage or use, and Apo-E will also carry residual lipids to the liver for excretion. The Apo-E protein known has three genetic forms that have different compositions. Apo-E3 is the most common form compared to other types of Apo-E2. Apo-E3 are less recognized by the cell surface, whereas ApoE4 binds more tightly to the receptor. One of these alleles each person will inherit one alleles from each parent. A person who has the same allele from each parent is called homozygous such as E2/E2 or E3/E3 or E4/E4. People who have different alleles are called heterozygous i.e. E2/E3 or E2/E4 or E3/E4. ApoE4 is associated with an increased risk of atherosclerosis. People with this genotype can tend to increase levels of triglycerides and LDL-C (bad cholesterol) significantly when the food consumed is dominated by high saturated fat [17]. The relationship between sex and genetic polymorphism plays a significant role in cardiovascular disease, especially in the incidence of genetic polymorphisms. This study shows that men are more at risk of experiencing Apo-E4 gene polymorphisms than women in the first generation of CHD in Bengkulu City. This research conducted in Northwest China which showed that men are more at risk of having the E4 allele genotype so they can also be more at risk of developing CHD. In addition, this study also shows that men who have the E4 allele have a 1.5-fold higher risk of developing carotid atherosclerosis [18].

Table 1. Frequency Distribution of Research Subjects [12,13]

	Characteristic Data	**Frequency**		**Total**
		N	**%**	
First Derivative Patients	CHD	21	50.00	42
	Non- CHD	21	50.00	
Sex	Male	16	33.30	42
	Female	26	66.70	
ABO blood group	A	10	19.00	42
	B	12	26.20	
	O	20	54.80	
ApoE Gene Genotype	E2	8	19.00	42
	E3	25	59.50	
	E4	9	21.40	
VEGF-A Gene Genotype	CC	18	42.90	42
	CG	8	19.00	
	GG	16	38.10	

Table 2. Gender Bivariate Analysis of Apo-E Gene Polymorphisms in First Derivatives of CHD and Non-CHD Patients

Subjects	Sex	Apolipoprotein E Gene Polymorphism					
		E2 and E3		E4			
		n	%	n	%	P-Value	OR(95% CI)
CHD	Female	10	83.30	2	16.70	0.009	17.50 (1.96-15.59)
	Male	2	22.20	7	77.80		
	Total	12	57.10	9	42.90		
Non-CHD	Female	13	92.90	1	7.10	1.000	2.16 (0.11-40.88)
	Male	6	85.70	1	14.30		
	Total	19	90.50	2	9.50		

Table 3. Analysis of ABO Blood Group of ApoE Gene Polymorphisms

First Derivative Patients	ABO Blood Group	Apolipoprotein E Gene Polymorphism							
		E2 and E3		E4					
		n	%	n	%	Total n	Total %	P-Value	OR(95% CI)
CHD	Non O	3	12.50	7	87.50			0.002	38.50 (2.91-508.46)
	O	9	84.60	2	15.40				
	Total	12	57.10	9	42.90	21	100		
Non-CHD	Non O	12	92.90	1	7.10			1.000	0.46 (0.02-8.69)
	O	7	85.70	1	14.30				
	Total	19	90.50	2	9.50	21	100		

Table 4. Gender Bivariate Analysis of VEGF-A Gene Polymorphisms

First Derivative Patients	Sex	VEGF-A Gene Polymorphism							
		CG and GG		CC					
		n	%	n	%	Total n	Total %	P-Value	OR(95% CI)
CHD	Female	9	75.00	3	25.00			0.008	24.00 (2.06-27.96)
	Male	1	11.10	8	88.90				
	Total	10	47.60	11	52.40	21	100		
Non-CHD	Female	9	64.30	5	35.70			1.000	0.72 (0.10-5.16)
	Male	5	71.40	2	28.60				
	Total	14	66.70	7	33.30	21	100		

Table 5. Blood Type Bivariate Analysis of VEGF-A Gene Polymorphisms

First Derivative Patients	ABO Blood Group	VEGF-A Gene Polymorphism							
		CG and GG		CC					
		n	%	n	%	Total n	Total %	P-Value	OR (95% CI)
CHD	Non O	1	12,50	7	87,50			0,024	15,75 (1,42-174,24)
	O	9	69,20	4	30,80				
	Total	10	47,60	11	52,40	21	100		
Non-CHD	Non O	9	64,30	5	35,70			1,000	1,38 (0,19-9,96)
	O	5	71,40	2	28,60				
	Total	14	66,70	7	33,30	21	100		

The study also found that the Apo-E4 genotype was a risk factor for CHD and progressed more rapidly in patients under 50 years of age [19]. A higher male risk of developing CHD is also associated with smoking habits. The Framingham study has elucidated the mechanism that atherogenic lipoprotein cholesterol profiles in young adults is strongly associated with smoking habits. This fact that smoking cessation may be the most cost-effective approach to the preventing steps [14]. Apo-E gene polymorphisms with CHD are mediated through blood lipid and non-blood lipid pathways. The absence of Apo-E in macrophages triggers atherosclerosis without changing cholesterol in plasma [7]. Populations of European ancestry showed that the genotype of the E4 allele increased the risk of death although the specific explanation for male or female remains unclear with no associations with both sexes reported [20,21]. Analysis of blood type revealed that respondents with a history of first-generation CHD patients were dominated by the E4 allele in the non-O blood group (87.50%). In addition, the analysis also showed that there was a significant relationship between blood type and Apo-E gene polymorphisms in the first generation of CHD patients in Bengkulu City (p: 0.002). The group in the first descendant of non-CHD showed no significant relationship between blood type and Apo-E gene polymorphism in the first descendant of patients with Non-CHD in Bengkulu City (p: 1,000). Respondents with blood type non-O first-generation CHD sufferers have a 38.50 times higher risk of experiencing Apo-E4 polymorphisms compared to first-generation non-CHD patients. This is known from the Odds Ratio value obtained at 38.50 (95% CI 2.91-508.46), while the first derivative of non-CHD is 0.46 (95% CI 0.02-8.69) (Table 3). Since the ABO blood group system was found, several studies have investigated the relationship between the ABO blood group system and various diseases. One of the relationships found was the relationship between ABO blood group and pulmonary thromboembolism (PE), ischemic heart disease, and deep vein thrombosis [22]. In this study we found that the relationship between Non-O blood type and Apo-E gene polymorphisms in the first generation of CHD patients in Bengkulu city is shown (p: 0.002). This is related to previous research on risk factors for CHD which resulted that non-O blood groups, namely blood types A, B, and AB had a higher risk of CHD events than blood group O which had a lower risk of cardiovascular disease such as CHD [16]. Research that has been reported before supported that non-O blood types are at higher risk of developing CHD. Individuals with non-O blood type, i.e. blood group A or B are at risk of up to 1.6 times higher risk of thromboembolic events, compared to individuals with O blood type. Blood type A is associated with a higher risk of heart failure, atherosclerosis, and hyperlipidemia than individuals with blood type O. with blood group O. In addition, blood type A is associated with a higher risk of atopy compared with blood group O [22]. The comparison results show that blood type A is associated with a higher risk of hyperlipidemia compared to blood group B. Meanwhile, blood group B has a higher risk of myocardial infarction than blood type O. This is partly due to the presence of low von Willebrand. Factor (vWF) in blood group O. Expression of A or B antigens is the result of an allelic combination of genetic variants in the ABO gene on chromosome 9 (9q34.2) [23].

Our study also found that there are indications that men have a greater risk than women, especially in non-O blood types, A, B, and AB. Non-O blood type is associated with major cardiovascular risk factors and an increased rate of cardiovascular events. Individuals with non-O blood type are at risk of developing CHD [24]. This found was supported with the other study that has been shown the relationship between blood type and CHD risk was not altered by age, alcohol consumption, smoking status, physical activity, or history of diabetes [25]. The other study also published an epidemiological analysis and shown non-O blood groups showed consistent increases in serum cholesterol levels compared with O blood groups. total circulating levels and LDL cholesterol, as well as phytosterols as risk factors for atherosclerosis [26].

In addition, in the relevant literature, it is explained that blood type O is protective while blood type A has a dangerous risk in the incidence of cardiovascular disease [27]. Type O blood is also associated with lower levels of several other lipid properties, including HDL-C, total cholesterol, ApoA, and ApoB. The association of the ABO gene with total cholesterol and LDL-C is consistent with the GWAS results, so some of the effects of blood type on cardiovascular disease may be caused by LDL-C [28].

3.3. Prevalence of Sex and Blood Types of VEGF-A Gene Polymorphisms

This study revealed that there was a significant relationship between gender and VEGF-A gene polymorphisms in the first descendant of CHD patients in Bengkulu City (p: 0.008). The VEGF-A gene polymorphism in the first descendant of patients with CHD was dominated by women (75.00%) with CG and GG genotypes, while the CC genotype was dominated by males (88.90%). In the non-CHD first-generation group, there was no significant relationship between sex and VEGF-A gene polymorphisms in the first-generation non-CHD patients in Bengkulu City (p: 1,000). The Odds ratio value shows that men have a 24 times higher risk of experiencing VEGF-A polymorphisms with the CC genotype compared to women (Table 4). The results showed that non-O blood type was significantly related to the VEGF-A gene polymorphism in the first descendant of CHD patients in Bengkulu City (p: 0.024). The Odds ratio value indicates that respondents with non-O blood type have a 15.75 times higher risk of developing VEGF-A gene polymorphisms with CC genotype than respondents with O blood type (Table 5). One of the risk factors that cannot be modified is gender, genetics, and age. The results of this study indicate that there is a significant relationship between the male sex and VEGF-A gene polymorphism (genotype CC) in the first generation of CHD patients in Bengkulu City (p: 0.02) [12]. The mechanism underlying VEGF-A can be a risk factor for CHD, namely glycoproteins as the main regulator of the angiogenesis process that can increase endothelial cell migration to areas experiencing hypoxia. Various previous studies revealed that several genetic variations of genes with angiogenic function play an important role in the development of atherosclerosis. Vascular Endothelial Growth Factor (VEGF) has angiogenic and atherosclerotic properties that are associated with several human diseases, such as diabetic retinopathy and CHD. The results of the genetic analysis study found that the allele frequency for the gene rs2010963 was higher in men than women [10,29].

The research before reported that the CC genotype has a higher likelihood of suffering from multi-vessel coronary atherosclerosis as well as an increased risk of CHD. Meanwhile, the possible interaction between blood type and known risk factors for thrombosis (gender, body mass) [4]. The results of this study have the same results as the study conducted by which showed that the CC genotype of the VEGF-A gene polymorphism (rs2010963) had susceptibility to CHD compared to other genotypes [10]. Identification of blood groups with a risk of atherosclerosis revealed that non-O blood groups (blood groups A, B) had a higher risk of developing atherosclerosis, especially in men compared to men with blood type O [22]. Based on the results of the Chi-Square test in this study showed that there was a relationship between VEGF-A gene polymorphism and non-O blood type in the first descendant of CHD patients (p: 0,024). The same conclusion also reported from the other research conducted by the Genome-Wide Association Study which found that there was a relationship between ABO blood group and cardiovascular disease [30]. Other studies have reported that a person with blood type A are at higher risk of developing hyperlipidaemia and atherosclerosis which are risk factors for CHD compared to blood group O. Individuals with blood type O are at lower risk of myocardial infarction than individuals with blood type B [31]. Although it is not only blood type that can influence atherosclerosis. Other habits such as smoking or consuming fatty foods can also be risk factors[32].

4. Conclusions

This study concludes that men in the first derivatives of patients with CHD have a higher risk of experiencing Apo-E4 and VEGF-A gene polymorphisms (CC genotype). Meanwhile, non-O blood types in the first derivatives of patients with CHD in Bengkulu City have a higher risk of experiencing Apo-E4 and VEGF-A gene polymorphisms (CC genotype). The limited number of samples was one of limitation of this research. In addition, further research is still needed regarding other risk factors that play a role in the incidence of atherosclerosis in the first generation of CHD.

Acknowledgements

This research has been conducted in Research Laboratory of Faculty of Medicine and Health Sciences, Bengkulu University. Besides, M. Yunus Hospital and Basic Science Laboratory, Biology Department in Faculty of Mathematics and Sciences, Bengkulu University also have been supported this research.

Ethical Approvals

The ethical clearance of this study was permitted by the Bengkulu University Health Research Ethics Committee number 97/UN30.14.9/LT/2022.

Declaration of Interest Statement

The authors assert that they have no conflicts of interest.

References

1. R. Bengkulu. (2018). Bengkulu Province Report RISKESDAS 2018. Ministry of Health of the Republic of Indonesia. 1–527.
2. Y. Wang, Q. Huang, J. Liu, Y. Wang, G. Zheng, L. Lin H. Yu, W.Tang, Z. Huang. (2017). Vascular endothelial growth factor A polymorphisms are associated with increased risk of coronary heart disease: A meta-analysis. Oncotarget. 8: 30539–30551. doi:10.18632/oncotarget.15546
3. W.Q. Ma, Y. Wang, X.Q. Han, Y. Zhu, N.F. Liu. (2018). Association of genetic polymorphisms in vascular endothelial growth factor with susceptibility to coronary artery disease: A meta-analysis. BMC Medical Genetics. 19: 1–12. doi:10.1186/s12881-018-0628-3
4. M. Zhao, I. Vaartjes, I. Graham, D. Grobbee, W. Spiering, K. Klipstein-Grobusch. (2017). Sex differences in risk factor management of coronary heart disease across three regions. Heart. 103: 1587–1594. doi:10.1136/heartjnl-2017-311429
5. F.A. Yousuf, and M.P. Iqbal. (2015). Apolipoprotein e (Apo E) gene polymorphism and coronary heart disease in Asian populations. Pakistan Journal of Pharmaceutical Sciences. 28: 1439–1444.
6. A.M. Minihane, L. Jofre-Monseny, E. Olano-Martin, and G. Rimbach. (2007). ApoE genotype, cardiovascular risk and responsiveness to dietary fat manipulation: Symposium on molecular basis for diseases. Proceedings of Nutrition Society. 66: 183–197. doi:10.1017/S0029665107005435
7. A.D. Marais. (2019). Apolipoprotein E in lipoprotein metabolism, health and cardiovascular disease. Pathology. 51: 165–176. doi:10.1016/j.pathol. 2018.11.002
8. W. Ma, Y. Wang, X. Han, Y. Zhu, and N. Liu. (2018). Association of genetic polymorphisms in vascular endothelial growth factor with susceptibility to coronary artery disease : a meta – analysis. BMC Medical Genetics. 1–12.
9. L. Li, Y. Pan, L. Dai, B. Liu, and D. Zhang. (2016). Association of genetic polymorphisms on vascular endothelial growth factor and its receptor genes with susceptibility to coronary heart disease. Med Sci Monit. 22: 31–40. doi:10.12659/MSM.895163
10. X. Han, L. Liu, J. Niu, J. Yang, Z. Zhang. (2015). Association between VEGF polymorphisms (936c/t, -460t/c and -634g/c) with haplotypes and coronary heart disease susceptibility. International Journal of Clinical and Experimental Pathology 8: 922–927.
11. M.S. Dahlan. (2010). Sample Size and Sampling Methods in Medical and Health Research. Edition 3. Jakarta: Salemba Medika. [Indonesian]
12. A.F. Anwari, N. Lestari, M. Asteria, S. Sipriyadi, I. Fahri, E. Yunita. (2021). VEGF-A Gene Polymorphism (rs2010963) in the First Inheritance of Coronary Heart Disease (CHD) Patients. BIOEDUSAINS: Journal of Biology and Science Education. 4: 325–335. doi:10.31539/bioedusains. v4i2.2630.
13. A.R.S. Prayogo, E. Yunita, R. Yolanda, I. Fahri, N. Lestari, M. Asteria et al. (2022). Carotid intima-media thickness in the first descendant of coronary artery disease patients with Apolipoprotein-E4 genotype. Bali Medical Journal. 11: 1774–1779. doi:10.15562/ bmj.v11i3.3798
14. G. Gallucci, A. Tartarone, R. Lerose, A.V. Lalinga, A.M. Capobianco. (2020). Cardiovascular risk of smoking and benefits of smoking cessation. Journal of

Thoracic Disease. 12: 3866–3876. doi:10.21037/jtd. 2020.02.47
15. M. Nadeem, S.S. Ahmed, S. Mansoor, and S. Farooq. (2012). Risk factors for coronary heart disease in patients below 45 years of age. Pakistan Journal of Medical Sciences. 29: 91–96. doi:10.12669/pjms. 291.2828
16. S. Li, C.M. Schooling. (2020). A phenome-wide association study of ABO blood groups. BMC Medicine. 18: 1–11. doi:10.1186/s12916-020-01795-4
17. L. Wu, Y. Zhang, H. Zhao, G. Rong, P. Huang, F. Wang, T. Xu. (2022). Dissecting the Association of Apolipoprotein E Gene Polymorphisms With Type 2 Diabetes Mellitus and Coronary Artery Disease. Frontiers in Endocrinology (Lausanne). 13: 1–8. doi:10.3389/fendo.2022.838547
18. W. Ma, L. Zhang, L. Luo, S. Zhang, S. Yang, H. Yao, L. Zhang, X. Lu, W. Feng. (2022). Effect of Apolipoprotein E ε4 Allele on the Progression of Carotid Atherosclerosis Through Apolipoprotein Levels. Pharmgenomics and Personalized Medicine. 15: 653–661. doi:10.2147 /PGPM. S367471
19. L.S. Ang, R.P. Cruz, A. Hendel, D.J. Granville. (2008). Apolipoprotein E, an important player in longevity and age-related diseases. Experimental Gerontology. 43: 615–622. doi:10.1016/j.exger.2008.03.010
20. D.C. Ewbank. (2002). Mortality differences by APOE genotype estimated from demographic synthesis. Genetic Epidemiology. 22: 146–155. doi:10.1002 /gepi.0164
21. F.J. Wolters, Q. Yang, M.L. Biggs, J. Jakobsdottir, S. Li, D.S. Evans, J.C. Bis, T.B. Harris, R.S. Vasan, N.R. Zilhao, *et al*. (2019). The impact of APOE genotype on survival: Results of 38,537 participants from six population-based cohorts (E2-CHARGE). PLoS One. 14: 1–13. doi:10.1371/journal.pone. 0219668
22. H.E. Groot, L.E.V. Sierra, M.A. Said, E. Lipsic, J.C. Karper, P. Van Der Harst. (2020). Genetically determined ABO blood group and its associations with health and disease. Arteriosclerosis, Thrombosis, and Vascular Biology. 830–838. doi:10.1161/ATVBAHA. 119.313658
23. X. Ni, C. Bai, C. Nie, L. Qi, Y. Liu, H. Yuan, X. Zhu, L. Sun, Q. Zhu, Y. Li, *et al*. (2021). Identification and replication of novel genetic variants of ABO gene to reduce the incidence of diseases and promote longevity by modulating lipid homeostasis. Aging (Albany NY). 13: 24655–24674. doi:10.18632/aging.203700
24. M. Paquette, R. Dufour, and A. Baass. (2018). ABO blood group is a cardiovascular risk factor in patients with familial hypercholesterolemia. Journal of Clinical Lipidology. 12: 383-389. doi:10.1016/j.jacl.2017.12. 001
25. D. Kamimura, L.R. Cain-Shields, D. Clark, A.A. Oshunbade, K.E. Ashley, C.S. Guild, P.D. Loprinzi, R. Newton, M.J. Blaha, T. Suzuki, *et al*. (2021). Physical Activity, Inflammation, Coronary Artery Calcification, and Incident Coronary Heart Disease in African Americans: Insights From the Jackson Heart Study. Mayo Clinic Proceedings. 96: 901–911. doi:10.1016/j.mayocp.2020.09.042
26. C.J. MacDonald, A.L. Madika, G. Severi, A. Fournier, and M.C. Boutron-Ruault. (2021). Associations between smoking and blood-group, and the risk of dyslipidaemia amongst French women. Scientific Reports. 11: 1–7. doi:10.1038/s41598-021-94239-9
27. S.B. Abegaz. (2021). Human ABO Blood Groups and Their Associations with Different Diseases. Biomed Research International. doi:10.1155/2021/6629060
28. S.R. Stowell, and C.P. Stowell. (2019). Biologic roles of the ABH and Lewis histo-blood group antigens part II: thrombosis, cardiovascular disease and metabolism. Vox Sanguinis. 114: 535–552. doi:10.1111/vox.12786
29. P. Douvaras, D.G. Antonatos, K. Kekou, S. Patsilinakos, G. Chouliaras, A. Christou, A. Andrikou, E. Kanavakis. (2009). Association of VEGF gene polymorphisms with the development of heart failure in patients after myocardial infarction. Cardiology. 114: 11–18. doi:10.1159/000210189
30. N.B. Larson, E.J. Bell, P.A. Decker, M. Pike, C.L. Wassel, M.Y Tsai, J.S. Pankow, W. Tang, N.Q. Hanson, K. Alexander, *et al*. (2016). ABO blood group associations with markers of endothelial dysfunction in the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis. 251: 422–429. doi:10.1016/j.atherosclerosis.2016.05.049
31. U.E. Nydegger, W.A. Wuillemin, F. Julmy, B.J. Meyer, and T.P. Carrel. (2003). Association of ABO histo-blood group B allele with myocardial infarction. Eur J Immunogenet. 30: 201–206. doi:10.1046/j.1365-2370. 2003.00390.x
32. T. Febriyanto, G. Baruara, and R. Ardhiana. (2023). Description of Blood Hemoglobin Level in Vape Users in the Hexohm Community Bengkulu City in 2021. B-Icon Proceeding. 2: 1–4.