

GENOMIC VARIANTS AND EPIDEMIOLOGY OF ATHEROSCLEROSIS – WORLDWIDE CORRELATION ANALYSIS AND UTILIZATION OF ATHEROSCLEROSIS GENE VARIANTS FOR IDENTIFICATION OF DRUG TARGET CANDIDATES WITH BIOINFORMATICS APPROACH

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ГЕНОМНИ ВАРИАНТИ И ЕПИДЕМИОЛОГИЯ НА АТЕРОСКЛЕРОЗАТА – СВЕТОВЕН КОРЕЛАЦИОНЕН АНАЛИЗ И ИЗПОЛЗВАНЕ НА ГЕНЕТИЧНИ ВАРИАНТИ, АСОЦИИРАНИ С АТЕРОСКЛЕРОЗА, ЗА ИДЕНТИФИЦИРАНЕ НА КАНДИДАТИ ЗА ЛЕКАРСТВЕНИ ЦЕЛИ С ПОМОЩТА НА БИОИНФОРМАТИЧЕН ПОДХОД

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Abstract:

Introduction: Atherosclerosis (AS) is a major risk factor for cardiovascular disease (CVD), which is the leading cause of death worldwide. Genetic factors are an integral factor in the cause of AS. This study aims to correlate epidemiological data, prevalence and mortality of AS worldwide with Single Nucleotide Polymorphisms (SNPs) associated with susceptibility and severity in different populations. Hence, utilized the gene

variants to discover potential drug targets using bioinformatics. **Material and methods:** This study used a secondary data-driven bioinformatics approach to analyze the correlation between genetic variants associated with AS and epidemiological data from different regions of the world. Genetic data were obtained from the GWAS Catalog, while epidemiological data were collected from WHO and Our World in Data. The analysis was performed by filtering SNPs based on several criteria, namely the missense variant category, significant p-value $< 10^{-5}$, and prediction of variant impact using PolyPhen (Possibly Damaging) and SIFT (Deleterious). The correlation between allele frequency and AS prevalence and mortality was analyzed using the Pearson test. The drug target identification process was carried out through the DrugBank database. **Results:** The results of the study obtained three missense variants – rs11466653 in *TLR10*, rs2296172 in *MACF1* and rs6025 in *F5*. Variant rs11466653 has a role in the pathogenesis of AS with analysis using SNPnexus which shows the possibility of damaging (PolyPhen) and deleterious (SIFT) properties in addition to the results of the correlation test significant to the global prevalence and mortality of AS ($p < 0.05$). Due to the challenge of drugging all potential target genes, our study was only able to identify the *F5* gene as a viable target. The *TLR10* gene is a molecular target for immunomodulators such as hydroxychloroquine, chloroquine, eritoran, and resatorvid (TAK-242) to reduce inflammation and regulate immune responses. In addition, tacrolimus as an immunomodulator shows potential in overcoming chronic inflammation associated with AS through a mechanism involving the *MACF1* gene. **Conclusion:** The findings in this study will encourage efforts to improve AS diagnosis and early treatment, as well as increase public awareness of the importance of genetic factors and lifestyle in preventing this disease. This study can also be a reference for further research focusing on genetic intervention and development of personalized therapy for AS. Therefore it can provide broader benefits for global public health.

Key words: atherosclerosis, *TLR10*, *MCF1*, *F5*, rs11466653, rs2296172, rs602

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Резюме.

Въведение: Атеросклерозата (АС) е основен рисков фактор за сърдечно-съдовите заболявания (ССЗ), които са водеща причина за смърт в световен мащаб. Генетичните фактори са неразделна част от причините за АС. Целта на това проучване е да се съпоставят епидемиологичните данни, разпространението и смъртността от АС в световен мащаб с единичните нуклеотидни полиморфизми (SNP), свързани с предразположеността и тежестта в различни популации. Ето защо с помощта на биоинформатиката използвахме генетичните варианти за откриване на потенциални лекарствени цели. **Материал и методи:** В това проучване е използван биоинформатичен подход, базиран на вторични данни, за да се анализира корелацията между генетичните варианти, свързани с АС, и епидемиологичните данни от различни региони на света. Генетичните данни са получени от каталога GWAS, а епидемиологичните данни са събрани от СЗО и *Our World in Data*. Анализът е извършен чрез филтриране на SNP въз основа на няколко критерия, а именно категорията "missense" вариант, значима р-стойност $< 10^{-5}$ и предсказване на въздействието на варианта с помощта на PolyPhen (възможно увреждане) и SIFT (увреждане). Връзката между честотата на алелите и разпространението на АС и смъртността е анализирана с помощта на теста на Пиърсън. Процесът на идентифициране на лекарствени цели е извършен чрез базата данни DrugBank. **Резултати:** Резултатите от това проучване идентифицираха три "missense" SNP варианта – rs11466653 в *TLR10*, rs2296172 в *MACF1* и rs6025 в *F5*. Вариант rs11466653 показва значими корелации с глобалното разпространение на АС и смъртността ($p < 0,05$). SNP rs11466653 има роля в патогенезата на АС, като анализът с помощта на SNPnexus показва вероятно увреждащи (PolyPhen) и вредни (SIFT) свойства. Поради предизвикателството да се лекуват всички потенциални целеви гени, нашето проучване успя да идентифицира гена *F5* като жизнеспособна цел. Генът *TLR10* е молекулярна мишена за имуномодулатори като хидроксихлороквин, хлороквин, ериторан и резаторвид (TAK-242), които намаляват възпалението и регулират имунните реакции. Освен това такролимус като имуномодулатор показва потенциал за преодоляване на хроничното възпаление, свързано с АС, чрез механизъм, включващ гена *MACF1*. **Заключение:** Резултатите от това проучване ще насърчат усилията за подобряване на диагностиката и ранното лечение на АС, както и за повишаване на обществената осведоменост относно значението на генетичните фактори и начина на живот за предотвратяване на това заболяване. Това проучване може да бъде и отправна точка за по-нататъшни изследвания, фокусирани върху генетичната намеса и разработването на персонализирана терапия за АС. Следователно то може да осигури по-широки ползи за общественото здраве в световен мащаб

Ключови думи: атеросклероза, *TLR10*, *MCF1*, *F5*, rs11466653, rs2296172, rs602

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INTRODUCTION

AS is a global health problem that causes serious complications such as coronary heart disease, stroke, and peripheral artery disease [1, 2]. This condition is characterized by the accumulation of plaque on the walls of the arteries, which causes narrowing and hardening of the arteries, reducing blood flow, and increasing the risk of cardiovascular events [3, 4]. According to data from the World Health Organization (WHO), cardiovascular disease, which is mostly caused by AS, is the leading cause of death worldwide, with around 17.9 million deaths each year [5]. The main risk factors for AS include unhealthy lifestyles such as smoking, a diet high in saturated fat, and lack of physical activity [6]. However, genetic factors also play an important role in a person's susceptibility to this disease [7]. Identification of genetic variants that potentially increase the risk of AS can provide important insights into the molecular mechanisms underlying this disease and help in the development of more effective prevention and treatment strategies [8]. Genome-Wide Association Studies (GWAS) is an approach used to identify genetic variants associated with complex diseases such as AS [9]. By analyzing data from large populations around the world, GWAS allow researchers to identify genetic variants associated with increased risk of AS [10]. Data from GWAS provide insight into genetic diversity across populations and how these variants may influence disease risk [11]. Recent research has identified several genes that play key roles in AS. For example, the *Low-Density Lipoprotein Receptor (LDLR)* gene, which encodes a receptor for LDL cholesterol, plays a role in the uptake of cholesterol from the blood [12]. Variations in this gene can increase blood levels of LDL cholesterol, a major risk factor for AS [13]. In addition, the *Apolipoprotein B (APOB)* gene, which encodes a key protein in LDL particles, and the *Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9)* gene, which regulates the number of LDL receptors on the surface of liver cells, also play a role in cholesterol metabolism and contribute to the risk of AS [14, 15].

The GWAS catalog has great potential in identifying pathogenic variants associated with AS. Through this method, researchers can identify associations between specific genetic variants and characteristics or diseases in large populations [16, 17]. Using the GWAS catalog, researchers can access data from a variety of published GWAS studies, allowing the identification of genetic variants that contribute to the risk of AS [10]. Its benefits include the identification of pathogenic variants that help understand the pathogenic mechanisms of the disease, the development of new, more effective therapies, the use of Mendelian randomization analysis to distinguish causal relationships in epidemiologi-

cal studies, and the development of AS risk prediction tools based on individual genetic profiles. Thus the GWAS catalog is a very valuable tool in AS research, helping to understand the causes of the disease and develop better therapies [18, 19]. Bioinformatics plays an important role in research on the correlation of AS gene variants with epidemiology worldwide [12]. Using bioinformatics analysis techniques, researchers can identify associations between specific genetic variants and the risk of AS. This analysis involves processing large-scale genetic data from multiple populations to find patterns that may not be immediately apparent. Thus, bioinformatics helps understand the pathogenic mechanisms of disease, identify potential therapeutic targets, and strengthen the relationship between genetic risk factors and the epidemiology of AS. This research can provide valuable insights that can be used for the development of more effective strategies for the prevention and treatment of AS [14, 15].

This study aims to identify genetic variants that potentially increase susceptibility to AS disease using data from various GWAS studies worldwide [20, 21]. By integrating genomic data from various populations, we hope to identify significant genetic variants and understand how genetic factors contribute to the risk of AS. This study is expected to make a significant contribution to the prevention and treatment of AS, as well as improve our understanding of the genetic mechanisms underlying this disease. Through this study, we also strive to develop more accurate diagnostic tools and more personalized treatment strategies. By knowing the genetic variants that increase the risk of AS, we can screen high-risk individuals early and provide timely interventions. In addition, a deeper understanding of these genetic mechanisms may help in the development of new drugs that can target specific molecular pathways involved in AS, thus providing new hope for the prevention and treatment of this disease in the future.

MATERIAL AND METHODS

This research method uses a bioinformatics approach and uses secondary data sources. Data collection was carried out retrospectively to analyze the correlation between genetic variants associated with AS and the epidemiology of this disease in various regions of the world. As well as the use of AS gene variants to identify candidate drug targets with a bioinformatics approach.

Epidemiological and Genetic Data

AS genetic data collected from the GWAS Catalog genome database downloaded on (08 November 2024) https://www.ebi.ac.uk/gwas/efotraits/EFO_0003914. Epidemiological data obtained from trusted sources such as WHO and *Our World in Data* downloaded on

(08 November 2024). Then analyzed by removing duplications and eliminating with missense and p-value 10^{-5} inclusion criteria. The results of the inclusion can be seen in Table 1. In addition, the results of gene variants downloaded from the GWAS Catalog database were further analyzed using the SNPnexus platform, which utilizes various prediction algorithms such as PolyPhen-2, and SIFT to evaluate the potential impact of genetic variants on protein function. Variant frequency data in the 1000 Genomes Project downloaded via Haploreg V42 downloaded on (08 November 2024), the allele frequencies of genetic variants in continental populations were evaluated Europe (EUR), Africa (AFR), East Asia (EAS), South Asia (SAS) and America (AMR) (Fig. 1).

Statistical Analysis

Pearson correlation was used for prevalence and mortality of AS, and variant allele frequencies. Data were evaluated using the “cor. Test” function of the “stats” package of the R programming language. After that, and after passing the Bonferroni correction

p-value threshold, r , r^2 , p-value, and 95% CI were obtained. All plots were created using the “ggplot2” graphical package. This package only considers p-values ≤ 0.05 as statistically significant. Correlation test is one of the statistical methods used to measure the strength and direction of the linear relationship between two variables. The correlation coefficient, usually denoted by r , shows the extent to which changes in one variable are followed by changes in the other variable. The correlation coefficient value ranges from -1 to 1. r -value approaching 1 indicates a strong positive correlation, a r -value approaching -1 indicates a strong negative correlation, and a r -value approaching 0 indicates no significant linear relationship between the two variables.

Identification of Drug Target Candidates

This study was conducted to identify drug candidates that can be proposed for drug repurposing, by targeting genes that play an important role in the molecular mechanisms of AS. Analysis using DrugBank resulted in a number of relevant drugs based on their

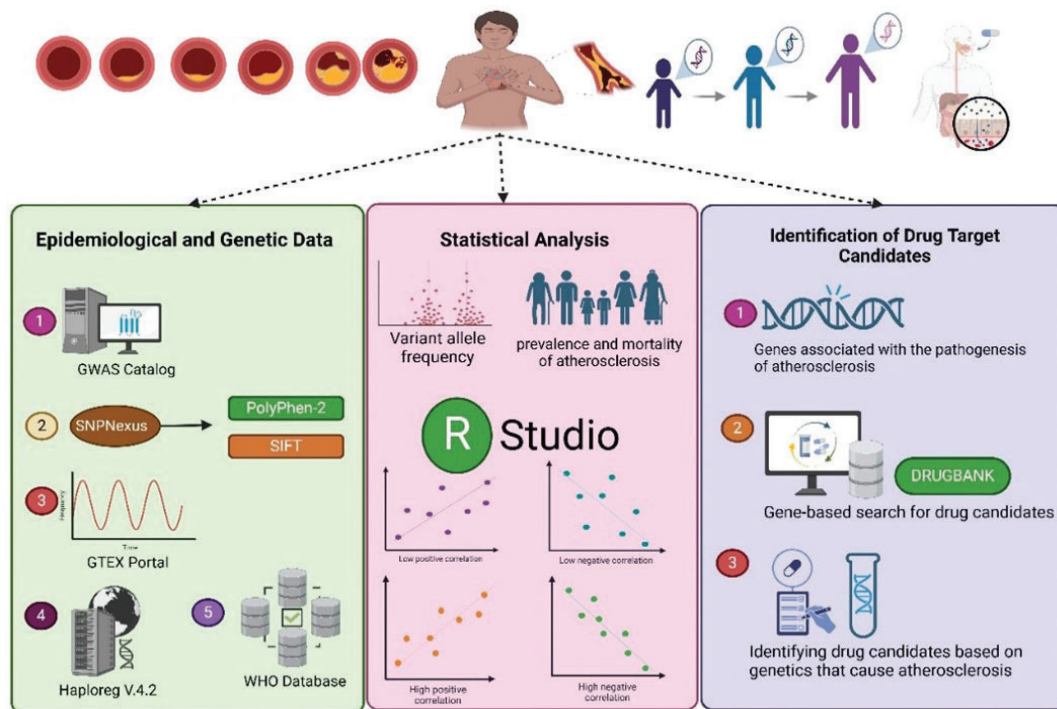


Fig. 1. Research methods flowchart, Created at <https://BioRender.com>

Table 1. Missense Variant Data and p-value 10^{-5} Gene Categories that have the potential to cause AS disease

Gene	SNPs	Variant Category	P-Value
<i>TLR10</i>	rs11466653	<i>missense_variant</i>	2.00E-07
<i>MACF1</i>	rs2296172	<i>missense_variant</i>	1.00E-06
<i>F5</i>	rs6025	<i>missense_variant</i>	2.00E-12

pharmacological interactions and molecular mechanisms with these genes.

RESULTS

Gene variants that have the potential to cause AS

The results of the study by downloading data from the GWAS catalog obtained 276 SNPs related to AS <https://www.ebi.ac.uk/gwas> after elimination using missense variants and a p-value category of 10^{-5} , 3 genes and 3 SNPs were directly associated with an increased risk of AS (Table 1). In addition to using the inclusion criteria, we also used further analysis with the SNPnexus platform, which utilizes various prediction method algorithms such as PolyPhen-2, and SIFT to evaluate the potential impact of genetic variants on protein function [22]. These algorithms analyze changes caused by mutations based on amino acid conservation across species (PolyPhen-2), the effects of nucleotide changes on protein structure and function (SIFT), and additional predictions based on biochemical properties or functional domains of the protein [23]. In (Table 2), the results of the analysis using the SNPnexus platform with the polyphen method are shown which is used to predict the impact of genetic variants on protein function. This method analyzes genetic mutations, especially amino acid changes to determine the potential damage caused by these mutations to the structure and function of the resulting protein [24]. There are three genetic variants (SNPs) that affect three main genes, namely *MACF1*, *F5*, and *TLR10*. These variants occur at specific positions in the genome and cause amino acid changes in the resulting protein. Each mutation is assessed based on its predicted impact score on protein function, which is classified as whether the mutation is benign or potentially damaging [25].

The SIFT method is used to predict the impact of genetic mutations on protein function by assessing the conservation of amino acid positions among different species [26]. In this study, mutations that occur at highly conservative positions (i.e. positions that maintain certain amino acids in many species) tend to be considered more detrimental to protein function, because changes at these positions are more likely to disrupt protein structure or function [26]. Conversely, mutations that occur at more variable positions (where amino acid changes are more common in evolution) are more likely to be accepted without significantly affecting protein function. The results of the analysis using the SNPnexus database with the SIFT method can be seen in Table 3.

Identification of gene expression in AS disease

Our results of the study on gene expression validate three genes, namely *TLR10*, *MACF1*, and *F5* genes that have been filtered strictly using the GTEx portal database <https://www.gtexportal.org/> (accessed on November 08, 2024). The goal of the step of knowing gene expression is to evaluate these genetic variants with gene expression profiles in many body tissues, which helps identify tissues that are relevant to the development of AS. The *TLR10* gene, which plays a role in immune and inflammatory responses, shows varying levels of expression across human tissues, with high expression observed in lymphoid tissues such as lymph nodes and spleen (Figure 2) [27]. Lymph nodes and spleen play important roles in the immune system and contribute to the process of AS through various mechanisms. Lymph nodes act as filters for body fluids, identifying and absorbing pathogens such as bacteria and viruses. When infection or inflammation occurs, the immune system is triggered to react, which can affect the process of AS. The spleen, on the other hand, produces and stores white blood cells that play a role in fighting infections.

Table 2. Prediction of the impact of genetic variants on proteins using the polyphen method

Variation ID	Chromosome	Position	Variants	Transcript	Wild AA	Mutant AA	Score	Prediction
rs2296172	chr1	39370145	A/G	ENST00000372915	M	V	0.001	<i>Benign</i>
rs2296172	chr1	39370145	A/T	ENST00000372925	M	L	0.02	<i>Benign</i>
rs6025	chr1	169549811	C/T	ENST00000367796	R	Q	0.6	<i>Possibly Damaging</i>
rs11466653	chr4	38774614	A/G	ENST00000308973	M	T	0.5	<i>Possibly Damaging</i>

Table 3. Prediction of the impact of genetic variants on proteins using the SIFT method

Variation ID	Chromosome	Position	Variants	Transcript	Wild AA	Mutant AA	Score	Prediction
rs2296172	chr1	39370145	A/G	ENST00000289893	M	V	0.09	<i>Tolerated</i>
rs2296172	chr1	39370145	A/T	ENST00000289893	M	L	1	<i>Tolerated</i>
rs6025	chr1	169549811	C/T	ENST00000367796	R	Q	0	<i>Deleterious</i>
rs11466653	chr4	38774614	A/G	ENST00000308973	M	T	0	<i>Deleterious</i>

These white blood cells produce cytokines that contribute to inflammation, an important factor in the formation of atherosclerotic plaques. In addition, lymph nodes and spleen help in the transport of antigens to larger immune areas, such as the thymus and bone marrow, which can trigger an excessive immune response and worsen the condition of AS. Modulation of the immune response by these two organs may also influence the formation of atherosclerotic plaques, since an excessive immune response can lead to chronic inflammation, a major factor in the development of AS. Figure 3 shows the expression of the *MACF1* gene in various human tissues where the highest gene expression is located in the lung. The *MACF1* gene is involved in microstructural stability and endothelial function. Endothelial cells are found in the lung [28]. Endothelial cells are a type of cell that lines the inside of blood vessels and play a critical role in maintaining blood vessel function and gas exchange. In the lung, endothelial cells line the pulmonary plexus, which is an important

part of the respiratory system [29]. The results of this study showed *high levels of MACF1* gene expression in lung and arterial tissues. High expression in these tissues suggests a role for *MACF1* in maintaining the structural integrity of cells, which is essential for normal endothelial cell function and blood flow regulation [17].

The *F5* gene, known as Factor V Leiden, is highly expressed in the liver, which is the primary site of clotting factor synthesis. This high expression indicates its critical role in coagulation processes (Figure 4). The *F5* gene, which encodes clotting factor V, is highly expressed in the liver and plays a key role in blood clotting. Factor V Leiden is a mutation in the *F5* gene that increases the risk of blood clot formation. In the context of AS, high expression of factor V in the liver may contribute to the formation of arterial plaque because factor V plays a role in the coagulation pathway that can trigger inflammation and platelet aggregation. Chronic inflammation and platelet aggregation are two major factors in the development of AS, so the *F5* gene,

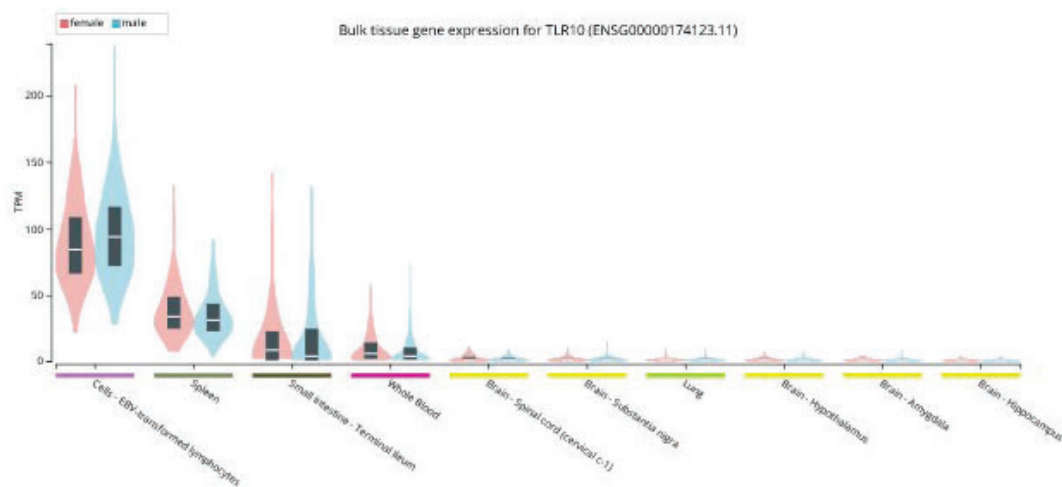


Fig. 2. Gene expression *TLR10* in human body tissues

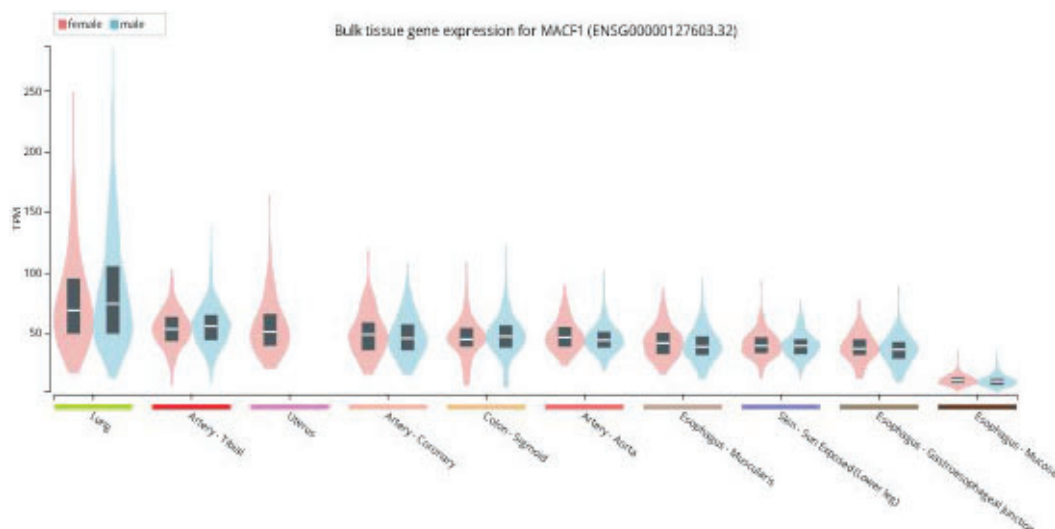


Fig. 3. *MACF1* gene expression in human body tissues

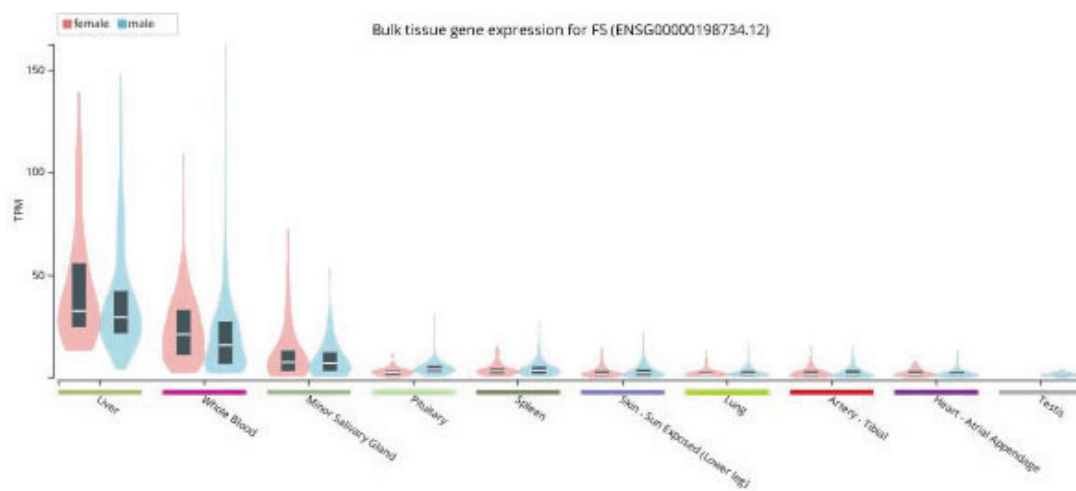


Fig. 4. *F5* gene expression in human body tissues

which functions in blood clotting, may influence the risk and progression of AS.

Frequency of alleles of genes that have the potential to cause AS in populations worldwide

We used the Haploreg genomic database version 4.2 to map the distribution of allele frequencies of each gene in different populations. The frequencies of alleles associated with AS were taken from genetic databases such as Haploreg version 4.2 <https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php> (accessed on November 11, 2024). Detection of the allele frequency of each variant is intended to confirm the allele frequency in populations from several continents (Africa, America, Asia, and Europe).

Description: The “Gene” column specifies the name of the analyzed gene, while “SNPs” (Single Nucleotide Polymorphisms) represents genetic variations involving a single nucleotide (Table 4). The “post (hg38)” column indicates the position of the SNP on the GRCh38/hg38 human genome reference build. The “Reference” and “Alternate” columns denote the nucleotide present in the reference genome and the alternate variant, respectively. The “1000 Genomes Phase 1 Frequencies (N)” provides allele frequencies of the alternate variant across different populations, including AFR (African), AMR (American), ASN (Asian), and EUR (European). The “Sequence constraint” column indicates evolution-

ary pressures on the genomic sequence, assessed using tools like GERP (Genomic Evolutionary Rate Profiling) and SiPhy (statistical methods to detect selective constraints). Finally, the “dbSNP functional annotation” describes the functional impact of the variant, such as “missense,” which refers to a mutation that alters the amino acid sequence of the encoded protein.

The results of this study evaluated three genetic variants associated with the *TLR10*, *MACF1*, and *F5* genes, each with potential functional implications based on their annotation and frequency distribution in the global population. The rs11466653 variant in the *TLR10* gene is located at position 38,774,614 (hg38), with reference allele A and alternative allele G. This variant has a minor allele frequency (MAF) of 0.03 in African (AFR), 0.16 in American (AMR), 0.1 in Asian (ASN), and 0.04 in European (EUR) populations. Based on dbSNP functional annotation, this variant is missense and is located in a region bounded by high conservation scores (GERP and SiPhy), indicating its potential impact on protein function. In the *MACF1* gene, the rs2296172 variant at position 39,370,145 (hg38) has a reference allele A and an alternative allele G, with MAFs of 0.02 (AFR), 0.21 (AMR), 0.16 (ASN), and 0.19 (EUR). Similar to the previous variant, this variant is also missense and shows high conservation value, indicating possible biological relevance to protein structure or function. The *F5* gene shows the

Table 4. Results of SNP Variants, Genes, Locations, Alleles, and Population Allele Frequencies Found

Gene	SNPs	post (hg38)	Reference	Alternate	1000 Genomes Phase 1 Frequencies (N)				Sequence constraint		dbSNP functional annotation
					AFR	AMR	ASN	EUR	by GERP	by SiPhy	
<i>TLR10</i>	rs11466653	38774614	A	G	0.03	0.16	0.1	0.04	Yes	Yes	missense
<i>MACF1</i>	rs2296172	39370145	A	G	0.02	0.21	0.16	0.19	Yes	Yes	missense
<i>F5</i>	rs6025	169549811	T	C	1	0.99	1	0.99	Yes	Yes	missense

rs6025 variant at position 169,549,811 (hg38), with the reference allele T and the alternative C. This variant has a nearly constant frequency in all populations (AFR 1.0, AMR 0.99, ASN 1.0, EUR 0.99). As a highly conservative missense, this variant may have a significant role in biological processes related to the function of the *F5* gene, which was previously known to be involved in the coagulation pathway.

Epidemiology in various populations

Epidemiological data taken from the WHO Our World in Data database in 2024, reveal interesting dynamics related to the prevalence and mortality of AS disease across continents (Figure 6). In Africa, there were 287.1 deaths per 100,000 population with a prevalence of 10,100 cases. These figures reflect a major health challenge, despite serious commitments to raise awareness and address the disease. In Asia, mortality reached 228.5 per 100,000 population with a prevalence of 7,400 cases, indicating an urgent need for more effective health interventions in the region. Meanwhile, Europe recorded a mortality rate of 178.1 per 100,000

population with a prevalence of 7,300 cases, indicating progress in addressing cardiovascular disease, although it still requires continued attention. In America, data shows the highest mortality rate with 302.1 deaths per 100,000 inhabitants and a prevalence of 7,800 cases, indicating the urgency to improve prevention and treatment of AS in this region.

Correlation of allele frequencies in the world with prevalence and mortality in the world

The results of the correlation test between the prevalence of AS in the entire world population with three different genetic variants, namely rs11466653, rs2296172, and rs6025. Each panel in Figure 7 displays a scatter plot with a regression line and the p-value can be seen in Figure 7. Based on the plot image, the p-value is $0.02827 < \alpha (0.05)$, which means that the correlation between the AS prevalence variable is significant with the rs11466653 allele frequency variable. The correlation coefficient value is known to be 0.9717297, which means that it has a positive relationship, namely if the rs1146653 allele frequency increases, the prevalence of

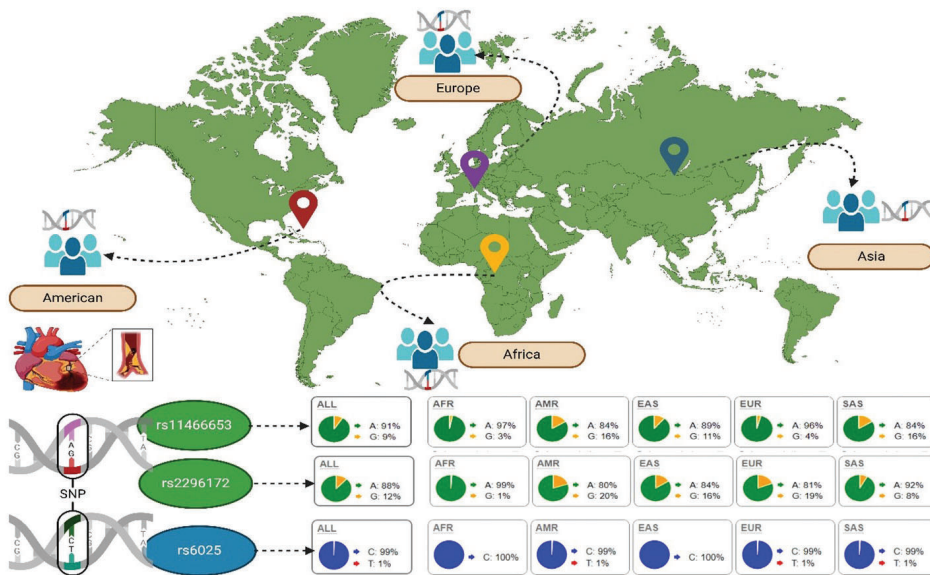


Fig. 5. Allele frequency distribution of three variants (rs11466653, rs2296172, rs6025) affecting *TLR*, *MACF1*, and *F5* among populations across continents



Fig. 6. Distribution of prevalence and mortality data. Data source: World Health Organization (2024)

AS disease increases, and this value is included in the strong relationship category. Based on the second panel in Figure 7, the p-value is $0.002903 < \alpha (0.05)$, which means that the correlation between the AS prevalence variable is significant with the rs2296172 allele frequency variable. The correlation coefficient value is known to be 0.9970972, which means that it has a positive relationship, namely if the rs2296172 allele frequency increases, the prevalence increases, and this value is included in the strong relationship category. Meanwhile, in the third panel (Figure 7), a p-value of $0.1573 > \alpha (0.05)$ was obtained, which means that the correlation between the prevalence variable and the rs6025 allele frequency variable is not significant. It is known that the correlation coefficient value is 0.842701, which means that there is

a positive relationship, namely that if rs6025 increases, mortality will increase, and this value is included in the strong relationship category.

The results of the correlation test between AS mortality in the entire world population with three different genetic variants, namely rs11466653, rs2296172, and rs6025. Each panel in the figure displays a scatter plot with a regression line and the p-value can be seen in Figure 9. Based on the results of the correlation test, the p value was obtained at $0.002871 < \alpha (0.05)$ which means that the correlation between the mortality variable is significant with the rs11466653 allele frequency variable. It is known that the correlation coefficient value is 0.9971286, which means that there is a positive relationship, namely that if the frequency of the rs11466653

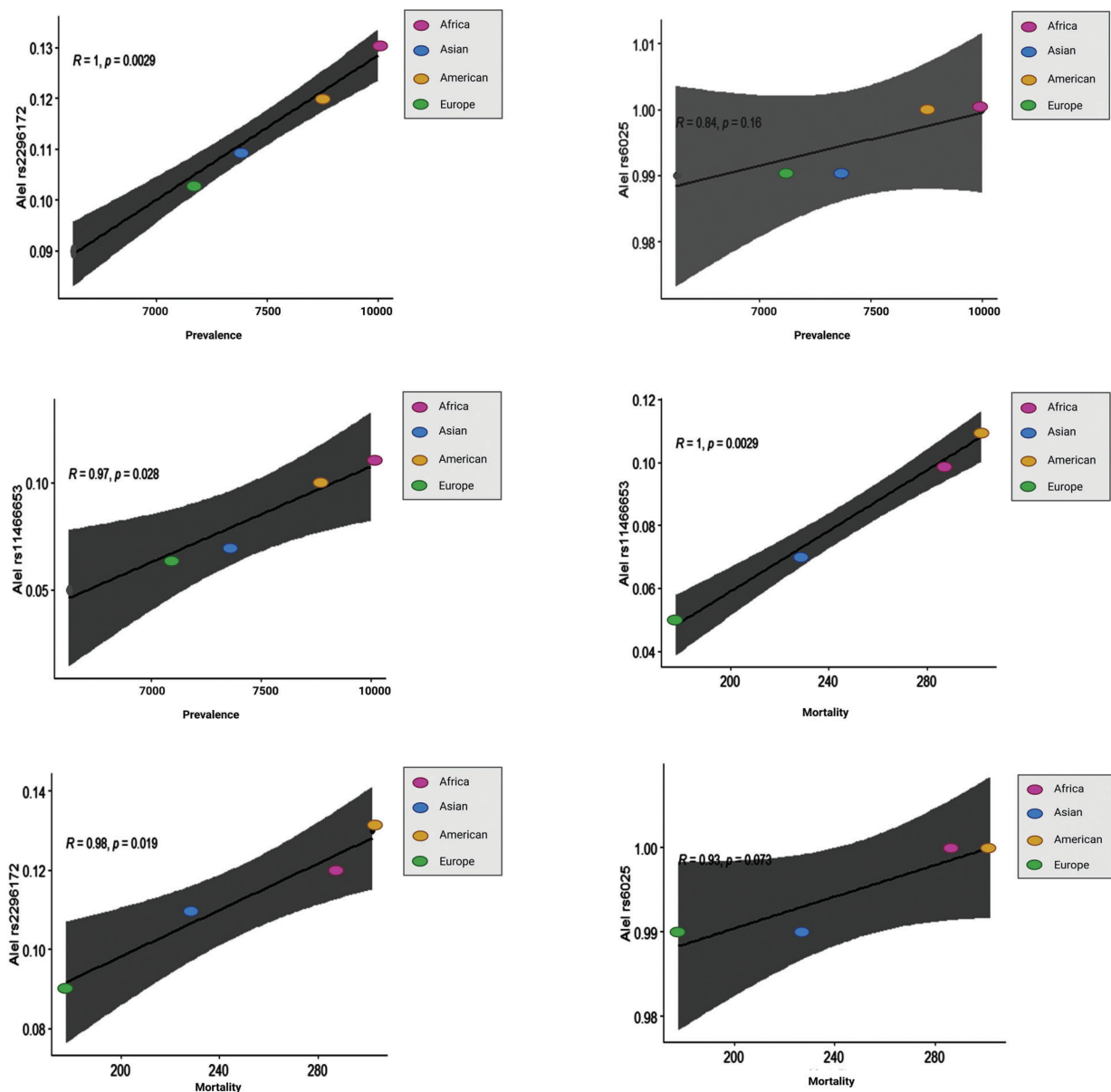


Fig. 7. Results of correlation test between variants rs11466653, rs6025, rs2296172 and prevalence, mortality of AS in the entire world population

allele increases, mortality increases, and this value is included in the strong relationship category. Based on the second panel (Figure 7), the p-value is $0.0192 < \alpha$ (0.05), which means that the correlation between the AS mortality variable is significant with the rs2296172 allele frequency variable. The correlation coefficient value is known to be 0.9807969, which means that it has a positive relationship, namely that if the rs2296172 allele frequency increases, the prevalence of AS increases and this value is categorized as having a strong relationship. While in the third panel (Figure 7), the p-value is $0.07262 > \alpha$ (0.05), which means that the correlation between the AS mortality variable is not significant with the rs6025 allele frequency variable. It is known that the correlation coefficient value is 0.9273821, which means that there is a positive relationship, namely that if the allele frequency at rs6025 increases, mortality will increase, and this value is included in the strong relationship category.

Identification of candidate drug targets

The study was conducted to identify drug candidates that could be proposed for drug repurposing, targeting the *TLR10*, *MACF1*, and *F5* genes, which have important roles in the molecular mechanisms of AS. Analysis using DrugBank resulted in a number of relevant drugs based on their pharmacological interactions and molecular mechanisms. The results of the study can be seen in Figure 8 and Table 5.

DISCUSSION

The results of the identification of drug candidates using DrugBank provide important insights into the potential for drug repurposing on the *TLR10*, *MACF1*, and *F5* gene targets, which have a central role in the pathophysiology of AS. These three genes are closely related to the mechanisms of inflammation, coagulation,



Fig. 8. The relationship between AS risk genes and drug repurposing to combat AS disease, Created in <https://BioRender.com>

Hydroxychloroquine Inhibits TLR activation which plays an important role in the body's immune response. Maintains the immune system from being excessive and reduces inflammation.

The mechanism of action of eritoran involves inhibiting LPS from binding to MD-2, a protein that works with TLR4 to recognize LPS. By inhibiting this, eritoran prevents the inflammatory signals normally activated by the MD-2/TLR4 complex, thereby reducing the inflammatory response.

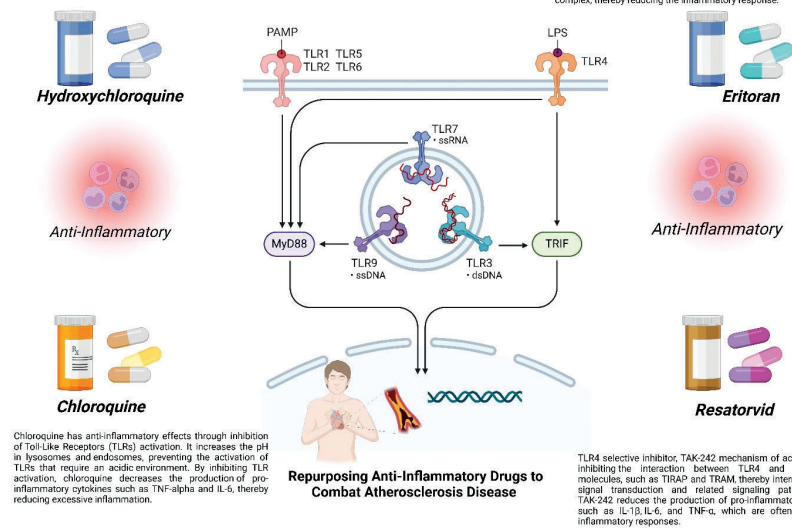


Fig. 9. Repurposing anti-inflammatory drugs to combat AS disease

Chloroquine has anti-inflammatory effects through inhibition of Toll-Like Receptors (TLRs) activation. It increases the pH in lysosomes and endosomes, preventing the activation of TLRs that require an acidic environment. By inhibiting TLR activation, chloroquine decreases the production of pro-inflammatory cytokines such as TNF-alpha and IL-6, thereby reducing excessive inflammation.

TLR4 selective inhibitor, TAK-242 mechanism of action involves inhibiting the interaction between TLR4 and its adaptor molecules, such as TRAP and TRAM, thereby interrupting TLR4 signal transduction and related signaling pathways. Thus, TAK-242 reduces the production of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α , which are often involved in inflammatory responses.

Table 5. AS drug target candidates based on F5, TLR and MACF1 gene variants

Drug	Disease Indications	Target Gene	Mechanism	Potential AS drug candidate	Clinical trials	DrugBank No./ PMID
Drotrecogin alfa	Severe Sepsis	F5	Activation of protein C, which inhibits factors Va and VIIIa, reduces excessive coagulation.	Thrombus Inhibitors	Phase III	DB00055
Thrombomodulin Alpha	Thrombolytic Agents	F5	Accelerates the activation of protein C through thrombomodulin, inhibiting excessive coagulation.	Thrombus Inhibitors	Phase III	DB05777
Thrombin	Coagulation Factors	F5	Functions as an essential cofactor for the prothrombinase activity of factor Xa which results in the activation of prothrombin to thrombin.	Anticoagulants	Phase IV	DB11300
Protein C	Vitamin C Deficiency	F5	Functions as an essential cofactor for the prothrombinase activity of factor Xa which results in the activation of prothrombin to thrombin.	Anticoagulants	Phase III	DB11312
Hydroxychloroquine	Lupus and rheumatoid arthritis	TLR	Inhibits TLR activation which plays an important role in the body's immune response. Maintains the immune system from being excessive and reduces inflammation.	Anti-inflammatory	Phase IV	DB01611
Chloroquine	Malaria	TLR	Chloroquine has anti-inflammatory effects through inhibition of Toll-Like Receptors (TLRs) activation. It increases the pH in lysosomes and endosomes, preventing the activation of TLRs that require an acidic environment. By inhibiting TLR activation, chloroquine decreases the production of pro-inflammatory cytokines such as TNF-alpha and IL-6, thereby reducing excessive inflammation.	Anti-inflammatory	Phase III	DB00608
Eritoran	Sepsis	TLR	TLR Antagonists which works by inhibiting the interaction between lipopolysaccharide (LPS) and Toll-like Receptor 4 (TLR4). The mechanism of action of eritoran involves inhibiting LPS from binding to MD-2, a protein that works with TLR4 to recognize LPS. By inhibiting this, eritoran prevents the inflammatory signals normally activated by the MD-2/TLR4 complex, thereby reducing the inflammatory response.	Anti-inflammatory	Phase III	DB04933
Resatorvid/ TAK-242	Sepsis	TLR	TLR4 selective inhibitor, TAK-242 mechanism of action involves inhibiting the interaction between TLR4 and its adapter molecules, such as TIRAP and TRAM, thereby interrupting TLR4 signal transduction and related signaling pathways. Thus, TAK-242 reduces the production of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α , which are often involved in inflammatory responses.	Anti-inflammatory	Phase II	DB05943
Tacrolimus	Immunomodulator	MACF1	Tacrolimus, as a calcineurin inhibitor, reduces T cell activity and the release of pro-inflammatory cytokines such as IL-2 and TNF- α .	Anti-inflammatory	Phase II	38774214

and cellular dynamics that underlie the development and complications of AS, such as plaque formation, plaque instability, and thrombosis.

F5 gene (Coagulation Factor V)

The *F5* gene plays a role in the coagulation pathway as an essential cofactor in the formation of the prothrombinase complex, which mediates the conversion of prothrombin to thrombin [30]. Excessive activation of this pathway increases the risk of thrombosis, especially in unstable atherosclerotic plaques. Regulation of *F5* activity is necessary to avoid complications such as myocardial infarction and stroke [31]. Drotrecogin Alfa (Activated Protein C) This drug is an active form of protein C that acts as an anticoagulation agent. The mechanism involves inactivation of factors Va (*F5* gene product) and VIIIa, thus inhibiting thrombin formation. In addition, Drotrecogin Alfa has anti-inflammatory effects by suppressing the release of pro-inflammatory cytokines, such as TNF- α and IL-6, which are relevant in AS [32]. Thrombomodulin Alfa This drug increases the conversion of protein C to its active form through binding to thrombin. Thrombomodulin Alfa indirectly inhibits factor Va activity, thereby preventing thrombus formation. Thrombin As a direct target of the coagulation pathway, thrombin can be modulated by thrombin-inhibiting agents. This mechanism helps reduce excessive thrombin activity, reducing the risk of hypercoagulability in AS. Protein C This protein is an endogenous anticoagulant that is activated by the thrombin-thrombomodulin complex [33]. Active protein C works by inactivating factors Va and VIIIa, reducing fibrin formation and the risk of thrombosis [34].

TLR10 Gene (Toll-Like Receptor 10)

TLR10 is an innate immune receptor that plays a role in pathogen recognition and regulation of inflammation. *TLR10* activation can trigger the release of pro-inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, which exacerbate chronic inflammation in AS. Modulation of *TLR10* activity is an important strategy to suppress vascular inflammation. Hydroxychloroquine and Chloroquine These drugs are known as immunomodulators that can inhibit the Toll-like receptor signaling pathway, including *TLR10* [35]. The mechanism is by disrupting the endosome/lysosome fusion required for *TLR10* activation. This inhibition reduces the release of pro-inflammatory cytokines, thereby reducing inflammation in the arterial wall. Eritoran As a TLR4 antagonist, Eritoran inhibits the activation of this receptor by lipopolysaccharide (LPS), which often directly affects the *TLR10* inflammatory pathway [36]. The effect is a decrease in the production of inflammatory cytokines and a reduction in the systemic inflammatory response. Resatorvid (TAK-242) This drug is a selective TLR4 inhibitor that works by interfering with the interaction of

TLR4 adapter proteins, thereby inhibiting the activation of downstream inflammatory pathways [37]. Although its primary target is TLR4, Resatorvid can affect *TLR10*-related inflammation systemically.

MACF1 Gene (Microtubule Actin Crosslinking Factor 1)

MACF1 is a key regulator in regulating the interaction between microtubules and actin filaments. In the context of AS, *MACF1* activity contributes to the migration and proliferation of vascular smooth muscle cells (VSMCs), which may exacerbate plaque formation and cause its instability [38]. Tacrolimus Tacrolimus is an immunosuppressive agent that works by inhibiting calcineurin, an enzyme essential for the activation of the NFAT (Nuclear Factor of Activated T-cells) pathway [39]. This pathway influences the migration and proliferation of VSMCs. By inhibiting the calcineurin-NFAT pathway, Tacrolimus can suppress *MACF1*-mediated cellular migration, thereby helping to stabilize atherosclerotic plaque [40]. In addition, Tacrolimus also has anti-inflammatory effects that can reduce the risk of AS progression [41].

CONCLUSION

The results of this study identified three SNP missense variants (rs11466653 in *TLR10*, rs2296172 in *MACF1*, and rs6025 in *F5*), with two of them (rs11466653 and rs2296172) showing significant correlations with global AS prevalence and mortality ($p < 0.05$). SNP rs11466653 has a role in the pathogenesis of AS, with analysis using SNP Nexus showing possibly damaging (PolyPhen) and deleterious (SIFT) properties. Analysis of candidate drug targets identified relevant therapeutic candidates: the *F5* gene can be targeted with coagulation inhibitors such as drotrecogin alfa, thrombomodulin alfa, thrombin, and protein C, which function as antithrombotic agents. The *TLR10* gene is a molecular target for immunomodulators such as hydroxychloroquine, chloroquine, eritoran, and resatorvid (TAK-242) to reduce inflammation and regulate immune responses. In addition, tacrolimus as an immunomodulator shows potential in overcoming chronic inflammation associated with AS through a mechanism involving the *MACF1* gene.

Ethics Approval: Medical And Health Research Ethics Committee (Mhrec) Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada – Dr. Sardjito General Hospital. Ethics Committee Approval Ref. No: Ke/Fk/1624/Ec/2024

Funding: This study was supported by Directorate of Research, Technology, and Community Service Ministry of Education, Culture, Research and Technology, Indonesia under the Grant No.107/ES/PG.02.00.PL/2024,0609.12/LL5-INT/AL.04/2024,115/PTM/LPPM UAD/VI/2024, 15 June 2024.

Acknowledgements: This study was supported by Directorate of Research, Technology, and Community Service Ministry of Education, Culture, Research and Technology, Indonesia.

No conflict of interest was declared

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