NASKAH PUBLIKASI

IDENTIFIKASI VARIAN GEN YANG BERPENGARUH PADA EFIKASI OBAT ANTIHIPERTENSI DENGAN MENGGUNAKAN PENDEKATAN BIOINFORMATIKA

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IDENTIFICATION OF GENE VARIANTS THAT AFFECT THE EFFICACY OF ANTIHYPERTENSION DRUGS USING A BIOINFORMATICS APPROACH

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ABSTRACT

Hypertension is a silent killer disease which shows blood pressure ≥ 140 mmHg/ 90 mmHg. Efficacy of providing the desired drug response. The pharmacological therapy used is diuretics, β-blockers, ACEI, ARB, and CCB. This study aims to determine gene variants that influence the therapeutic effects of antihypertensive drug use by utilizing a genomic database and to determine the frequency distribution of gene variants that influence the therapeutic effects of antihypertensive drugs in several populations in the world. This research was conducted using non-experimental methods using a bioinformatics approach. This research was carried out by combining data obtained from several genomic databases such as PharmGKB, Haploreg v4.2 and Ensembl. In this study, the inclusion criteria were single-nucleotide polymorphism (SNP) which influenced the treatment response to the efficacy of using antihypertensive drugs with missense SNPs, p-value < 0.05 and Level of Evidence (LOE) with levels 3 and 4. SNPs related to the efficacy of antihypertensive drugs were obtained from the PharmGKB database of 31 SNPs based on inclusion criteria. Then expanded using the Haploreg v4.2 database and obtained 7 SNPs that had missense variations. The global allele frequency distribution was viewed using the Ensembl database. The population that has a trend in the efficacy of antihypertensive drugs in Africa, America, Europe and South Asia with the highest frequency is the T allele with rs5522 which codes for the NR3C2 gene. Then in East Asia with high frequency, namely the G allele for rs699 and the T allele for rs5522 which codes for the AGT and NR3C2 genes. There are missense variants, namely rs4961, rs880054, rs699, rs5522, rs1799998, rs1799853, and rs1367117. Each encodes the ADD1, WNK1, AGT, NR3C2, CYP11B2, CYP2C9, and APOB genes which are related to the drugs hydrochlorthiazide, atenolol, enalapril, candesartan, and irbesartan

1. Introduction

In 2013, the World Health Organization (WHO) reported that 9.4 million out of 1 billion people in the world died from disorders related to cardiovascular disease (World Health

Organization, 2013). Hypertension occurs when the force of blood flow continuously compresses the blood vessels (Olin et al., 2018). According to the American Heart Association (AHA) quoted by Unger, hypertension is defined as blood pressure ≥140 mm Hg or diastolic blood pressure ≥ 90 mm Hg (Unger et al., 2020). Hypertension can be caused by several factors, one of which is genetic factors. Hypertension is caused by many gene factors. Certain genes are associated with systems involved in the mechanisms of hypertension, such as the immune and inflammatory systems, the renin-angiotensin-aldosterone (RAA) system, Gproteins or signal transduction pathway and ion channel systems (Angesti, 2018). One area of genetics that studies genetic variation in populations is called population genetics. Allele frequency is the percentage of an allele present in a population. The frequency of alleles in a population can change if there are evolutionary forces, namely factors that play a role in changing the frequencies of alleles and genotypes, including mutation, migration, non-random mating, and natural selection (Nur Khoiriyah et al., 2014). According to M. Fithrul Mubarok, efficacy is the maximum ability of a drug to produce a response. Or it can also be defined as the maximum effect that can be achieved with a drug. The goal of efficacy is to improve the patient's quality of life and reduce the risks of drug use. One way to find out how effective therapy is is by measuring blood pressure, effective blood pressure or the expected blood pressure target, namely ≤ 140 mmHg / 90 mmHg (Baroroh et al., 2023). One way to treat hypertension is through pharmacological treatment. Pharmacological treatment aims to achieve a better quality of life for patients, using antihypertensive drugs. Antihypertensive drugs that are often used are diuretics, β-blockers, ACEI, ARB, and CCB (JNC 8, 2014).

2. Materials and Methods

This research uses non-experimental methods. Researchers carry out the process of combining two or more data from various different database sources to identify gene variations that influence the efficacy of antihypertensive drugs. This was done by combining data obtained from the PharmGKB, Haploreg v4.2 and Ensembl databases as well as several literature as library sources to better support the research object by using the keywords gene variants, hypertension and efficacy.

1. Inclusion Criteria

- Hypertensive patients without comorbidities
- Five classes of antihypertensive drugs
- SNPs that influence treatment response to efficacy of use antihypertensive drugs with the keyword efficacy
- Significant P-value (<0.05)
- Level Of Evidance (LOE) with levels 3 and 4
- Missense SNP

2. Exclusion Criteria

- Drugs that do not have secondary data in the PharmGKB database

Tools and Materials

This study uses a tool, namely a set of Asus laptops with Windows 10 operational system, with the database used, namely PharmGKB, Haploreg v4.2, and Ensambl. This study uses materials, namely gene variant data associated with the efficacy of antihypertensive drugs seen from the PharmGKB, Haploreg v4.2 and Ensambl databases.

Data Analysis

a. Data on antihypertensive drugs uses the JNC 8 literature reference
b. SNPs that influence the efficacy of antihypertensive drugs were identified using the PharmGKB database (https://www.pharmgkb.org/) with enter one name of antihypertensive drug then take the SNP meet the inclusion criteria, namely SNPs that influence treatment response on the efficacy of using antihypertensive drugs that have Level Of The highest evidence (LOE) is level 3 and SNP is significant with p-value 0.05 is then validated using Haploreg v4.2 which is accessed on the link (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) to view SNPs with missense mutations and to validate the results are candidates Regulatory SNPs in antihypertensive drug efficacy-associated loci.
c. Frequency of alleles associated with treatment of hypertension in various populations obtained from the Ensembl database (https://asia.ensembl.org/index.html) for look at the distribution of allele frequencies in populations around the world.

3. Results and Discussion

A. Identification of Gene Variations that Affect the Efficacy of Antihypertensive Drugs

Efficacy is a monitoring in drug safety, it is said that the drug has efficacy if there is a decrease in blood pressure in hypertension sufferers ≤ 140 mmHg / 90 mmHg (Juwita et al., 2019). The antihypertensive drugs used in this study consisted of five classes of drugs, the first of which was the diuretic drug class including bendroflumethiazide, chlorthalidone, hydrochlorothiazide, and indapamide. The second is the β-blocker drug group including atenolol and metoprolol. The third is the ACEI drug class including captopril, enalapril, and lisinopril. Fourth, the ARB drug class includes eprosartan, candesartan, losartan, valsartan, and irbesartan. The fifth is the CCB drug class including amlodipine, diltiazem, and nitrendipine, of which antihypertensive drugs refer to JNC 8. The basis for these drugs is used in this study because it refers to previous research which used evidence base hypertension guidelines for treating adult patients who focuses on the 3 highest ranking questions through the Delphi modification technique.

The first genomic database used was PharmGKB. PharmGKB is a database to identify gene relationships with antihypertensive drugs that have the potential to be followed up as research material. From the research that has been conducted, 636 SNPs related to antihypertensive drugs were obtained through the PharmGKB database (accessed on January 25, 2024). Then from these 636 SNPs, 32 SNPs were obtained (Accessed on January 27, 2024) which were associated with the efficacy of antihypertensive drugs with a p-value <0.05 and Level Of Evidance (LOE) 3 and 4. The p value reflects the level of conformity or compatibility of the data with the null hypothesis, the value with p = 0.05 to take this point as a limit in assessing whether a deviation should be considered significant or not (Di Leo & Sardanelli, 2020). Level Of Evidence (LOE) is the level of evidence to classify research (Burns et al., 2015).

Table 1. SNPs with *p-value* significance <0.05 and *Level Of Evidence* (LOE) 3 and 4.

Varian	Signifikan	*p-value*	Level
rs1799752	Yes	*0,0001*	4
rs2246709	Yes	0,01	4
rs2740574	Yes	*0,02*	4
rs12346562	yes	0,0018	3
rs4961	yes	*0,003*	3
rs880054	yes	*0,01*	3
rs3758785	Yes	*0,0071*	3
rs3784921	Yes	*0,006*	3
rs7297610	Yes	*0,0196*	3
rs2269879	Yes	*0,01*	3

rs10792367	yes	*0,003*	3
rs699	Yes	0,008	3
rs7606603	Yes	*0,022*	3
rs11064426	Yes	*0,001*	3
rs2301339	Yes	*0,003*	3
rs5443	Yes	*0,001*	3
rs45545233	Yes	*0,0013*	3
rs1042714	Yes	0,0003	3
rs2106809	Yes	0,019	3
rs5186	Yes	0,022	3
rs699947	Yes	0,002	3
rs495828	Yes	*0,024*	3
rs5522	Yes	*0,003*	3
rs35068180	yes	0,009	3
rs1799998	yes	0,004	3
rs1045642	Yes	*0,03*	3
rs12721226	yes	*0,001*	3
rs1799853	Yes	0,036	3
rs5370	Yes	0,007	3
rs1367117	Yes	*0,004*	3
rs5186	Yes	*0,02*	3

SNPs were then developed through a second *database*, *Haploreg v4.2*. *Haploreg v4.2 database* is a database used to view SNPs with missense mutations and to validate genes that have been obtained in PharmGKB. Missense is a mutation that can cause impaired protein function and eventually lead to human disease (Petrosino et al., 2021). Missense SNPs are used because in missense SNPs there are changes in nitrogenous bases that will cause changes in amino acids. SNPs developed through the Haploreg v4.2 database resulted in 7 missense (accessed on February 14, 2024) that have a risk associated with antihypertensive drug efficacy.

Table 2. Variant risk allele encoding 8 genes

Variant	***p-value***	***Gen***	**Lokasi** ***Allele***	**Level**	**Alel** **Reff**	**Eff**
rs4961	*0,003*	*ADD1*	*Missense*	3	G	T
rs880054	*0,01*	*WNK1*	*Missense*	3	C	T
rs699	0,008	*AGT*	*Missense*	3	A	G
rs5522	*0,003*	*NR3C2*	*Missense*	3	C	T
rs1799998	0,004	*CYP11B2*	*Missense*	3	A	G
rs1799853	0,036	*CYP2C9*	*Missense*	3	C	T
rs1367117	*0,004*	*APOB*	*Missense*	3	G	A

Ref, reference; Eff, Allele Effect.

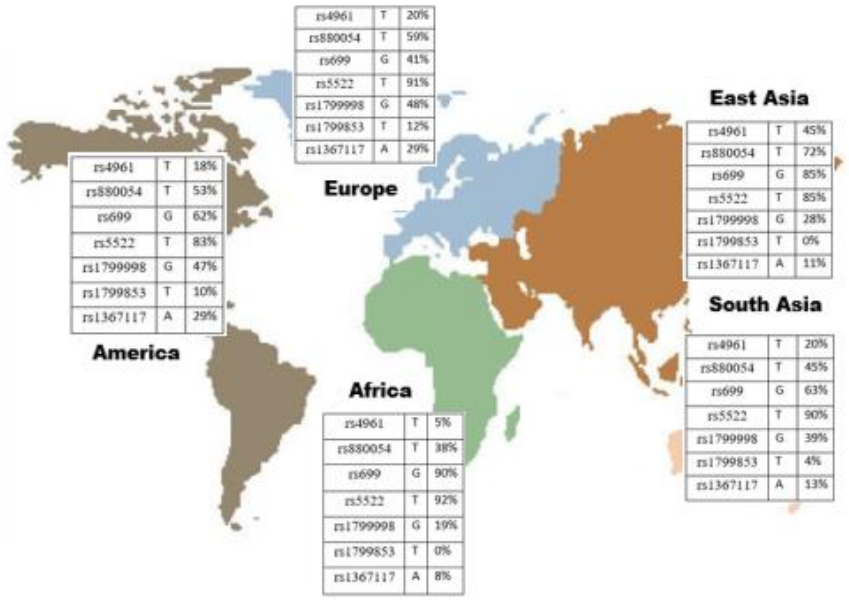
B. Allele Frequency of Gene Variants in Different Parts of the World

After identifying gene expression variants in antihypertensive drug efficacy, the allele frequencies in the world population were sought. The frequency of allelic variants was evaluated in world populations, such as Europe, America, East Asia, South Asia, and Africa (Figure 3). Allele frequencies were obtained from the Ensembl Genome Browser (accessed on February 3, 2024).

Table 3. Allele frequencies at each SNP in different parts of the world

SNP	Posisi	Gen	Lokasi	Alel		Frekuensi Alel (N)				
				Ref	Eff	AFR	AMR	EAS	EUR	SAS
rs4961	Chromosome 4:2904980	*ADD1*	*Missense*	G	T	0.049 (65)	0.174 (121)	0.452 (456)	0.205 (206)	0.200 (196)
rs880054	Chromosome 12:879392	*WNK1*	*Missense*	C	T	0.377 (499)	0.549 (381)	0.717 (723)	0.593 (597)	0.459 (449)
rs699	Chromosome 1:230710048	*AGT*	*Missense*	A	G	0.903 (1194)	0.635 (441)	0.853 (860)	0.412 (414)	0.636 (622)
rs5522	Chromosome 4:148436323	*NR3C2*	*Missense*	C	T	0.927 (1226)	0.827 (574)	0.854 (861)	0.916 (921)	0.905 (885)
rs1799998	Chromosome 8:142918184	*CYP11B2*	*Missense*	A	G	0.189 (250)	0.468 (325)	0.287 (289)	0.486 (489)	0.395 (386)
rs1799853	Chromosome 10:94942290	*CYP2C9*	*Missense*	C	T	0.008 (11)	0.099 (69)	0.001 (1)	0.124 (125)	0.035 (34)
rs1367117	Chromosome 2:21041028	*APOB*	*Missense*	G	A	0.078 (103)	0.287 (199)	0.115 (116)	0.298 (300)	0.133 (130)

AFR, Afrika ; AMR, Amerika ; EAS, Asia Timur ; EUR, Eropa ; SAS, Asia Selatan ; N, Jumlah Total Sampel ; Reff, Referensi ; Eff, Efek Alel AFR, AMR, EAS, EUR, SAS yang diambil dari *Ensembl*



Picture 1. Percentage of global allele distribution using the Ensembl database

From the picture above, it is known that the percentage of alleles associated with the efficacy of antihypertensive drugs in each continent is different. This is because the population and races on each continent are different. Different races on each continent result in changes in bases so that the genes on each continent are different.

In Africa, the frequency of the allele effect is high, namely the G allele on rs5522 which codes for the NR3C2 gene with a frequency of 92%. In America, the frequency of allele effects is high, namely the G allele on rs5522 which codes for the NR3C2 gene with a frequency of 93%. In Europe, the frequency of the allele effect is high, namely the G allele on rs5522 which codes for the NR3C2 gene with a frequency of 91%. In South Asia, the frequency of the allele effect is high, namely the G allele on rs5522 which codes for the NR3C2 gene with a frequency of 90%. And in

East Asia, the frequency of allele effects is high, namely the T allele and G allele respectively at rs699 and rs5522 and which code for the AGT and NR3C2 genes with a frequency of 85%. The frequency of allele effects in the entire population for each SNP can be different because the sample size used varies for each population. The aim of knowing the expression of each gene in various continents can be used to assist in the discovery and development of new drugs that are marketed throughout the world and for a more structured treatment of hypertension.

4. Conclusion And Suggestions

A. Conclusion

Based on the research that has been carried out, the author obtained conclusions from research regarding the Identification of Gene Variants on the Efficacy of Antihypertensive Drugs Using a Bioinformatics Approach, as follows:

1. There are 7 gene variants that influence the efficacy of antihypertensive drugs obtained from the genomic database, namely rs4961 (ADD1), rs880054 (WNK1), rs699 (AGT), rs5522 (NR3C2), rs1799998 (CYP11B2), rs1799853 (CYP2C9), and rs1367117 (APOB).
2. The distribution of SNPs in the world population shows different allele frequencies on each continent. This is due to several factors such as population, environment and living habits. The population with the highest frequency of efficacy trends for antihypertensive drugs in Africa, America, Europe and South Asia is the T allele with rs5522 which codes for the NR3C2 gene. Then in East Asia, the frequency is high, namely the G allele for rs699 and the T allele for rs5522 which codes for the AGT and NR3C2 genes.

B. Suggestions

Based on the research that has been carried out, the author provides suggestions from research regarding the identification of gene variants on the efficacy of antihypertensive drugs using a bioinformatics approach, as follows:

1. Research regarding side effects related to antihypertensive drugs that target genes can be further developed.
2. Data on the distribution of gene variations that cause the efficacy of antihypertensive drugs can be studied more widely in other populations in the world with the use of genomic databases.
3. It is hoped that the use of genomic databases for research using similar databases but with other medicines will also be useful for the development and discovery of treatments in the future.

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