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***Tujuan:*** Penelitian ini bertujuan mengetahui variasi polimorfisme gen ADRB2 posisi 16 dan 27 pada pasien asma di Jawa. ***Metode:*** Penelitian ini merupakan penelitian observasional menggunakan rancangan potong lintang yang dilakukan di lima rumah sakit di Daerah Istimewa Yogyakarta dan satu rumah sakit di Jateng selama satu tahun antara Juni 2015 sampai Mei 2016. Subjek penelitian adalah pasien dengan gangguan fungsi pernafasan yang menjalani pengobatan di poliklinik penyakit dalam di rumah sakit tersebut dan memenuhi kriteria inklusi dan eksklusi serta bersedia ikut dalam penelitian dengan menanda tangani surat pernyataan persetujuan. ***Hasil:*** Total pasien sebanyak 101 yang memenuhi kriteria inklusi dan eksklusi. Hasil yang diperoleh pada kodon 16 adalah, sebanyak 75 orang (74,26%) memiliki genom type Arg16, 10 orang (9,90%) Gly 16, dan 16 orang (15,84%) Arg16Gly. Pada kodon 27 hasil yang diperoleh adalah, sebanyak 65 orang (64,36%) memiliki genotype Gln 27, 8 orang (7,92 %) Glu 27, dan 28 orang (27,72%) Gln 27 Glu. ***Kesimpulan:*** Jumlah mutan pada kodon 16 lebih banyak (9,90%) dibanding kodon 27 (7,92%), dan tidak ada mutan kodon 16 bersama mutan pada kodon 27 pada seseorang.

**Kata Kunci**: Polimorfisme, ADRB2, Arg16Gly, dan Glu17Gln

***Aim:*** This study aims to determine variations in ADRB2 gene polymorphisms at positions 16 arginine to glycine (Arg16Gly) and at position 27 (glutamate acid to glutamine (Glu27Gln) asthma patients in Java. ***Method****:* A case cross sectional design was perfomed with 101 adult subject asthmatics from regions Yogyakarta and Central Java. The ADRB2-16 and ADRB2-27 polymorphisms were genotype by PCR-RFLP. ***Results:*** There are total 101 patients who fulfill the inclusion and exclusion criteria. The results obtained at codon 16 is, there are 75 people (74.26%) had Arg16 genotype, 10 people (9.90%) Gly 16, and 16 people (15.84%) Arg16Gly. At codon 27, the results obtained are, there are 65 people (64.36%) had genotype Gln 27, 8 people (7.92%) Glu 27, and 28 people (27.72%) Gln 27 Glu. ***Conclusion:*** The amount of the mutant codon 16 (9.90%)

is higher than mutant codon 27 (7.92%), but none of the patients had mutant at codon 16 and 27.

**Keywords:** Polymorphism, ADRB2, Arg16Gly, and Gln17Glu

## INTRUDUCTION

Asthma can affect children and adults, family history could be a trigger factor of asthma disease risk with different genes. Many genes are involved in the onset of asthma, such as adrenergic receptor β2 or ADRB2.[1,2] Genetic influences on drug response may occur in the phase of pharmacokinetics and pharmacodynamics. On pharmacodynamic phase there is an interaction between the drug and receptor because of the configuration receptor changing as the result of gene mutation.[3,4] ADRB2 gene is located on chromosome 5q31-32, reported to be polymorphic, related with asthma phenotype.[5,6] ADRB2 can be identified in the coding region of the gene in the 5 'and 3' have SNP more than 50[4] , and Some have claimed 80 SNP.[3] At the beginning of the discovery, there are nine polymorphisms, four of them are Arg16Gly, Gln27Glu, Val34Met, and Thr164Ile, which Arg16Gly and Gln27Glu are the most prevalent polymorphism.[7,8]

Mutation of a specific gene will alter the therapeutic response of asthma, mutation of Arg16Gly can provide various effects for different drugs[9], namely an increase in acute albuterol response[10,11], increase in risk of exacerbations [12,13] , and the long-term treatment response with short-acting β2-agonist decreases.[12] Patients with Glu 27 variant, the use of ACE inhibitor is more efficient than beta blockers in hypertension due to left ventricular hypertrophy.[14] In the 16-Gln Arg haplotype 27 gives a greater response than Gly16-Glu 27 on the use of salbutamol inhaler.[15]

Genotype variation is found in some country such as 16% of Arg16 in Caucasia and 25% in Afro- America [3,16] The distribution of Arg16 for White American is 39,3%, for Black American is 49,2%, and Chinese is 51,0%.[17] On codon 27, allel Glu27 is not found in China but it is found in Caucasia- America with the rate 15,4%, and 4,9% in Africa-America. [3,16]

## METHODS

Blood samples for genotype variation examination are collected from the asthmatic patients who are fulfilling the inclusion and exclusion criteria. Those patients sign an informed consent as their approval. As the research subject, there are 101 patients from 5 hospitals in *Daerah Istimewa Yogyakarta* (Yogyakarta Special Regency) and 1 hospital in Central Java. The study was approved by the Medical Research Ethics Committee of Faculty of Medicine, Gadjah Mada University.

### Identifikasi β2AR-16 (Arg16Gly)

Genomic DNA is obtained from 2 μl of blood, examination of polymorphism is determined by PCR-RFLP (Polymerase Chain Reaction - Restriction Fragment Length Polymorphism). Primers used for Arg16Gly and Gln27Glu are 5'-GCC TTC TTG CTG GCA CCC CAT -3 '(foward) and 5'-CAG CTC ACGGAA CTT GGC CAT G -3 '(reverse). Furthermore, the digestion (cutting) the DNA produced by PCR (168 bp) using enzyme Ncol, at 37 ° C for 2 hours. Enzyme Ncol cuts 22 bp from the end of the 3' on the right and limits 18 bp from the end of the 5' on the left. PCR analysis is done by electrophoresis using 4% agarose gel visualized by staining using ethidium bromide and viewed under UV light. The results of PCR-RFLP of β2-AR gene variant is 146 bp Arg16Gly to heterozygous Arg16 (Wild Type), 128 bp for heterozygous Gly16 (Mutants), and 146 bp and 128 bp for heterozygous Arg16Gly.[18]

### Identiifikasi β2AR-27 (Gln27Glu)

Identification Gln27Glu is using foward primer, reverse primer, and a sequence of equal treatment with Arg16Gly. Furthermore, digestion (cutting) the DNA produced by PCR (168 bp) using enzyme BbvI, at 37 ° C for 2 hours. PCR analysis is done by electrophoresis using 4% agarose gel visualized by staining using ethidium bromide and viewed under UV light. The results of PCR-RFLP of genes β2 Gln27Glu-AR variant are 105 bp and 63 bp for homozygot Gln27 (Wild Type), 168 bp for homozygous Glu 27 (Mutants), and 168 bp, 105 bp, and 63 bp for heterozygous Gln27Glu.[18]

## Data Analysis

The data genotype test results grouped by type genotype at each codons (16 and 27). Furthermore, the amount of each type of the genotypes Arg16Gly and Gln27Glu are percentaged and expressed in %.

## RESULTS

The results obtained at codon 16 is, there are 75 people (74.26%) had Arg16 genotype, 10 people (9.90%) Gly 16, and 16 people (15.84%) Arg16Gly. At codon 27, the results obtained are, there are 65 people (64.36%) had genotype Gln 27, 8 people (7.92%) Glu 27, and 28 people (27.72%) Gln 27 Glu.

## DISCUSSION

The research result shows in codons 16 and 27, each shows the type of wildtype, mutant, and heterozygous. Research result in Table 1, shows the amount of the distribution and percentage of each type of the genotypes. At codon 16, as much as 9.90% of the subject has a mutant genotype (Gly16), whereas mutant genotype at codon 27 (Glu27) is as much as 7.92%. The previous research result in Table 2 shows the distribution of the mutant genotype at codon 16 and 27 from some countries. In this research, mutation of genotype at codon 16 Arginine to glycine (Gly16) is found as much as 9.90%. The mentioned number is the smallest compared to Gly16 found in Oman's population (63%), Caucasian-American (62%), Turkey (60%), African-American (51%), and China (41%). At codon 27, Glutamine to Glutamate (Glu27) amounted to 7.92% smaller than Oman (75%), Caucasian-American (42%), Turkish (32%), African-American (21%), but higher than Chinese (7.20%).[19,20,21] From these distributions, Java, Indonesia genotype mutation that occurs at codon 27 is approaching Chinese.

In table 3 is shown the haplotype distribution of the survey population, as much as 47.52% is a combination of wild type- wild type allele, and several other types of haplotypes, but no mutant-mutant haplotypes. This result is different from what that was done by Balushi (2015), where the mutant-mutant haplotype amounted to 25%.[19] The difference in the genotype and haplotype variations between ethnic groups, may produce different effects on the use of the same

drug. Ferdinands research (2007) shows, the black population in America with genotype Arg16Arg, on the use of β2-agonists provides better result than those who have genotype Gly16Gly.[22]

## CONCLUSIONS

The amount of the mutant codon 16 (9.90%) is higher than mutant codon 27 (7.92%), but none of the patients had mutant at codon 16 and 27.

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Table 1. Genotype frequency of codons 16 and codons 27

| **Type of genotype** | **Codon 16** | | **Codon 27** | |
|---|---|---|---|---|
| | n | % | n | % |
| Wild Type | 75 | 74,26 | 65 | 64,36 |
| Mutant | 10 | 9,90 | 8 | 7,92 |
| Heterozygous | 16 | 15,84 | 28 | 27,72 |

Table 2. Comparative frequency of Gly16 and Gln27 polymorphisms in various ethnic groups reported in the literature.

| **Author and year of study** | **Population** | **Frequency of polymorphism (%)** | | **Total sample size** |
|---|---|---|---|---|
| | | **Gly16** | **Glu27** | |
| Aynacioglu et al, 1999 | Turkish | 60 | 32 | 104 |
| Xie et al, 1999 | African-American | 51 | 21 | 123 |
| Xie et al, 1999 | Caucasian-American | 62 | 42 | 188 |
| Xie et al, 1999 | Chinese | 41 | 7,2 | 104 |
| Balushi, 2015 | Oman | 63 | 75 | 316 |
| Present study, 2016 | Indonesia | 9,9 | 7,92 | 101 |

Table 3. Haplotype frequency of ADRB2 genetic polumorhisms (rs1042713/rs1042714

| Haplotype | | n | % |
|---|---|---|---|
| Arg16-Gln27 | Wild Type-Wild Type | 48 | 47,52 |
| Arg16-Gln27Glu | Wild Type-Heterozygous | 21 | 20,79 |
| Arg16-Glu27 | Wild Type-Mutant | 6 | 5,94 |
| Arg16Gly-Gln27 | Heterozygous-Wild Type | 11 | 10,89 |
| Arg16Gly-Gln27Glu | Heterozygous-Heterozygous | 3 | 2,79 |
| Arg16Gly-Glu27 | Heterozygous-Mutant | 2 | 1,98 |
| Gly16-Gln27 | Mutant-Wild Type | 8 | 7,92 |
| Gly16-Gln27Glu | Mutant-Heterozygous | 2 | 1,98 |