ORIGINAL ARTICLE

# Polymorphism of Gene ACE I/D and Family History of Hypertension as Predisposition of Hypertension

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**ABSTRACT**

**Introduction:** Hypertension is a polygenic disease that caused 45% of deaths. Various genes have been engaged with the pathogenesis of hypertension. One of these genes affects sodium homeostasis in the kidney, including the ACE I/D gene polymorphism. The present study aimed to investigate the relationship of family history of hypertension and ACE I/D gene polymorphism with the incidence of hypertension in coastal communities of Kendari City. **Methods:** The study was conducted using a case-control study design. The case group was hypertensive patients based on medical diagnostic by doctors, while the control group was healthy individuals without any records on hypertension. As many as 70 individuals residing in the coastal area of Kendari City were involved as samples of the study. Both case and control groups consisted of 35 individuals. Data collection techniques were carried out experimentally using the PCR-RFLP method. **Results:** The prevalence of I allele was found in individuals with a family history of hypertension (72.1%) as compared to the D allele (27.9%). The study also found a significant correlation between the family history of hypertension and ACE I/D gene polymorphism (p-value 0.001). However, there was no significant relationship between ACE I/D gene polymorphism and the incidence of hypertension in this population (p-value 0.631). **Conclusion:** Family history of hypertension was a risk factor for the incidence of hypertension. On the other hand, the polymorphism of ACE I/D gene was a protective factor towards the incidence of hypertension.
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**Keywords:** Family History of Hypertension, Polymorphism of ACE I/D Gene, Hypertension

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## INTRODUCTION

According to the World Health Organization (1), about 17 million people worldwide died each year due to cardiovascular diseases. Among which, approximately 9.4 million people died caused by its complication with hypertension every year. In Indonesia, the prevalence of hypertension has been reported to be 26.5%. Of this percentage, as many as 31.7% occurred in people aged 18 years and over. Based on the Integrated Disease Surveillance of the Community Health Center (Puskesmas), the non-communicable diseases hypertension and diabetes mellitus were included in the top 10 diseases in Southeast Sulawesi Province in 2015 (2). The report showed 37,036 cases of hypertension in 2012 and 46,465 cases in 2013. Moreover, records of hypertension patients in Kendari City was 6,856 cases in 2014 and increased about 8,550 cases in 2015 (3).

Based on the geographical data, three Community Health Centers (Puskesmas) actively serve in the coastal area of Kendari City that are Community Health Centre of Nambo, Abeli, and Mata. The case of hypertension has been in the top 5 of the 20 most diseases in these centers. Their records showed the increment of patients suffering from hypertension in 2015 which 729 people in Nambo, 1,394 in Abeli, and 955 people in Mata. In 2017, there was an increase in hypertension sufferers, namely 801 people in Nambo, 1,514 people in Abeli, and 1,025 people in Mata. In 2019, the number of patients at the Nambo Health Center decreased by 769 people, the Abeli Health Center increased to 1,112 people and the Mata Health Center was 1,102 people (4).

Family history of hypertension is an non-modified risk factor. Several studies have shown a strong correlation between family history and the prevalence of hypertension (5). Healthy and non-obese adult men with hypertensive parents would have a high chance of suffering high blood pressure as compared to adult men with normotensive parents. In addition, adult men with one hypertensive parent would probably have intermediate blood pressure (6).

The Renin-angiotensin-aldosterone system (RAAS) plays an important role in controlling blood pressure and

sodium homeostasis. Previous studies have reported RAAS polymorphism as a genetic determinant of essential hypertension (7,8). The renin-angiotensin-aldosterone system is a complex endogenous system involved with the regulation of components of arterial blood pressure, where activation and regulation is regulated mainly by the kidneys. Renin catalyzes the conversion of angiotensinogen to angiotensin I in the blood. Angiotensin I is then converted to angiotensin II by (ACE). Angiotensin II type 1 Receptor (AGTR1) with ACE mediation plays an important physiological role in regulating blood pressure blood. Blood contains angiotensinogen produced by the liver, which by the hormone Renin (produced by the kidneys) is converted to angiotensin I (a decapeptide not active). By ACE in the lungs, angiotensin I is converted to angiotensin II (Rudnichi et al, 2004). Angiotensin II mediated by AGTR1 have important responses for cardiovascular and renal function. Circulation of angiotensin II can increase blood pressure through pressor and volume effect. Pressor effects include direct vasoconstriction, stimulation of release catecholamines from the adrenal medulla, and centrally mediated increased activity sympathetic nervous system. Angiotensin II also stimulates aldosterone synthesis from adrenal cortex. This causes sodium and water reabsorption which increases plasma volume, total peripheral resistance, and ultimately affect blood pressure (9–12).

RAAS-produced Angiotensin Converting Enzyme (ACE) has been described as a crucial enzyme in the pathogenesis of hypertension (13–15). Hence, the ACE insertion/deletion polymorphism has a strong correlation to the RAAS polymorphism. On the other hand, some studies have shown controversial results between the ACE insertion/deletion polymorphism and the incidence of hypertension. Research by Alexander Vinsent reported that the presence of allele I in the ACE gene polymorphism was not significantly associated with hypertension in ischemic stroke patients (16). Similarly, it was reported that there was no association between ACE gene polymorphisms and essential hypertension in Japan (17).

Polymorphism of ACE I/D gene resulted in 3 genotypes, namely homozygous II, heterozygous ID, homozygous DD12. The correlation of ACE I/D gene polymorphism with either hypertension or ACE inhibitors response has been reported in some studies. The polymorphism also affects both serum ACE concentration and blood pressure. Studies showed that people with DD genotype had a double ACE concentration when compared with people with II genotype. On the other hand, people with ID genotype had either medium or moderate plasma ACE concentrations. Meanwhile, people with II genotype had a stable ACE concentration (18).

The polymorphism of the ACE I/D gene is associated strongly with a family history of coronary artery disease (CAD). Individuals with a history of hypertension have been shown to possess a higher prevalence of D allele than individuals without history. Hypertension is one of the risk factors for CAD and shows a higher prevalence in individuals with a history of hypertension (19). The correlation between family history of hypertension and ACE I/D gene polymorphism is crucially needed a better understanding, which can explain the onset of individuals with a family of hypertension. However, there has been little studies done on this topic. The present study was aimed to investigate the relationship of family history of hypertension and ACE I/D gene polymorphism with the incidence of hypertension in coastal communities of Kendari City, Indonesia.

## MATERIALS AND METHODS

### Materials

The material used in this study were the Wizard ® Genomic DNA Purification Kit (Promega) for extracting blood sample DNA, Go Taq ® Green Master Mix (Promega) for DNA amplification, Primers of I/D genes ACE: 5 'CTG GAG ACC ACT CCC ATC CTT TCT-3 'as forward and 5' CGT CAG AT-GAT GGC CAT CAC ATT-3' as reverse.

### Subjects

Case-control observational analytical study (case-control study) with experimental methods through a molecular biology approach. The study sample consisted of 35 cases and 35 controls. Criteria for the case group were hypertensive patients diagnosed with a doctor, aged 18-55 years, did not smoke, and did not drink alcohol. Sampling technique was purposive sampling. The control group criteria were subjects with normal blood pressure (normotensive). This study obtained ethical feasibility under the Health Research Ethics Committee of the College of Medicine, Halu Oleo University (Reference number: 031 /UN29.20/PPM/2020).

### DNA Extraction

Whole blood samples (300 µL) were transferred to each microtube and added with 900 µL of cell lysis solution. The mixture was incubated for 10 minutes to lyse the red blood cells. Blood samples then homogenized by turning back and forth 2-3 times and centrifuged at 13,000 x g for 1 minute at room temperature. After removing the supernatant, the pellet added with 300 µL of nuclei lysis solution and the solution was pipetted 5-6 times to lyse the white blood cells. Protein precipitation solution (100 µL) was added to the nuclear lysate and vortexed for 10-20 seconds. The mixture then centrifuged at 13,000 x g for 3 minutes at room temperature. Transferred supernatant to a new tube and added with isopropanol to 300 µL. The solution mixed gently until a visible mass of white thread-like strands of DNA observed. This solution then centrifuged at 13,000 g for 1 minute at room temperature. The suspension containing DNA further added with 100 µL of 70% ethanol. This

solution was cleaned by centrifugation at 13,000 g for 1 minute. Afterward, the supernatant discarded and the suspension was dried for 45-60 minutes at room temperature to evaporate the solvent. The suspension was added with 100 µL of DNA rehydration solution and incubated at 65°C for 1 hour. DNA samples then stored in a refrigerator at 2-8°C.

### DNA Amplification

The PCR mixture composition with a total volume of 12.5 µL contained 1 µl Natural Free Water (NFW), 7.5 µl Go tag green, 1 µl primer ACE forward 5 'CTG GAG ACC ACT CCC ATC CTT TCT-3', 1 µl reverse ACE primer 5 'GAT GGC CAT CAC ATT CGT CAG AT-3', and 2 µl template DNA. PCR amplification (thermocycler) was carried out at a temperature of 94°C for 5 minutes, followed by 30 cycles at 94°C for 1 minute, 67°C for 1 minute, 72°C for 2 minutes, and the last stage at 72°C for 4 minutes.

### Electrophoresis

Agarose gel electrophoresis was used to visualize the results of PCR using a Bio-Rad Horizontal MiniSub DNA electrophoresis system. Agarose gel prepared with a concentration of 2%. The electrophoresis was carried out at 100 volts for 30 minutes.

### Statistical Analysis

Isolation of ACE II, ID and DD receptor gene fragment was analyzed using the PCR fragments (PCR- RFLP) using primary nucleotides: 5 'CTG GAG ACC ACT CCC ATC CTT TCT-3 'as forward and 5' CGT CAG AT-GAT GGC CAT CAC ATT-3' as reverse, consisting of 3 genotypes The II genotype appeared as one band at 490 bp (homozygous). The ID genotype ID showed as two bands at 190 bp and 490 bp (heterozygotes). Furthermore, the DD genotype has appeared as one band at 190 bp (homozygote).

Statistical analysis was performed using the SPSS version 16.0 for Windows. The correlation was analyzed using the Chi-square test. Allele and genotype frequencies were calculated using the Hardy-Weinberg's formula: $[p^2 + 2pq + q^2 = 1]$, where $p^2$ was the frequency of homozygote A, 2pq was the heterozygote Aa, and $q^2$ was the frequency of homozygotes mutant (15).

## RESULTS

In both groups, there were significant age differences ($p < 0.05$). The 36-45-year-olds were most dominant in both groups. There were no subjects of hypertension at the age of 17-25 years, but at the age of 26-35 years, there were eight subjects with hypertension. The percentage of family history of hypertension found higher (82.9%) in the hypertension group as compared to the normotensive group (37.1%). In the hypertension group, it also found that the percentage of overweight (45.7%) and obesity (14.3%) BMI was higher than in the normotensive group (37.1% and 8.6%, respectively). Meanwhile, the HDL and LDL were not significantly different in both groups (Table I).

**Table I: Characteristics of subjects**

| Characteristics | Groups | | Total |
|---|---|---|---|
| | Hypertensive | Normotensive | |
| | n = 35 (%) | n = 35 (5) | n = 70 (%) |
| **Age** ( p* 0.019) | | | |
| 17–25 years | 0 (0) | 6 (17.1) | 6 (8.6) |
| 26–35 years | 8 (22.9) | 8 (22.9) | 16 (22.8) |
| 36–45 years | 17 (48.6) | 14 (40.0) | 31 (44.3) |
| 46–55 years | 10 (28.6) | 7 (20.0) | 17 (24.3) |
| **Sex** | | | |
| Male | 12 (34.3) | 11 (31.4) | 23 (32.9) |
| Female | 23 (65.7) | 24 (68.6) | 47 (67.1) |
| **Polymorphism of ACE I/D** | | | |
| II | 20 (57.1) | 18 (51.4) | 38 (54.25) |
| ID | 12 (34.4) | 13 (37.1) | 25 (35.75) |
| DD | 3 (8.6) | 4 (11.4) | 7 (10.0) |
| **Family History of Hypertension** ( p** 0.000) | | | |
| Positive | 29 (82.9) | 13 (37.1) | 42 (60) |
| Negative | 6 (17.1) | 22 (62.9) | 28 (40) |
| **BMI** | | | |
| < 18.5 kg/m$^2$ | 1 (2.9) | 0 (0) | 1 (1.4) |
| 18.5 – 22.9 kg/m$^2$ | 8 (22.9) | 7 (20) | 15 (21.4) |
| 23.0 – 24.9 kg/m$^2$ | 5 (14.3) | 11 (21.4) | 16 (22.9) |
| 25.0 – 29.9 kg/m$^2$ | 16 (45.7) | 13 (37.1) | 29 (41.4) |
| >30 kg/m$^2$ | 5 (14.3) | 3 (8.6) | 8 (11.4) |
| **HDL- cholesterol** ( p* 0.941) | | | |
| < 40 (mg/dL) | 13 (37.1) | 8 (22.9) | 21 (30) |
| 40-60 (mg/dL) | 18 (51.4) | 24 (68.6) | 42 (60) |
| ≥ 60 (mg/dL) | 4 (11.4) | 3 (8.6) | 7 (10) |
| **LDL- cholesterol** ( p* 0.600) | | | |
| < 100 (mg/dL) | 5 (14.3) | 5 (14.3) | 10 (14.3) |
| 100 – 129 (mg/dL) | 11 (31.4) | 13 (37.1) | 24 (34.3) |
| 130 – 159 (mg/dL) | 12 (34.3) | 12 (34.3) | 24 (34.3) |
| 160 – 189 (mg/dL) | 7 (20) | 5 (14.3) | 12 (17.1) |

* Statistically significant ($p < 0.05$).

Two bands at 490 bp and 190 bp has used as the basis for identification (Figure 1). The genotypic analysis showed that among 70 DNA amplified, 38 samples had II genotype, followed by genotypes of ID (25 samples) and DD (7 samples). The frequency of II genotype found as many as 54.3% with 76 alleles. Meanwhile, the frequency of ID and DD genotypes showed percentages of 35.7% and 10.0%, respectively (Table II). Heterozygote subjects owned one of each allele. Hence, 25 subjects with ID genotype had either I or D alleles. Similarly, 7 subjects with DD genotype with two D allele copies contributed 14 D alleles. These subjects shared 101 I alleles and 39 D alleles. The frequency of genotype II found higher compared to ID and DD genotypes. Similarly, the frequency of I allele was higher than the D allele. The Chi-square test showed a value of

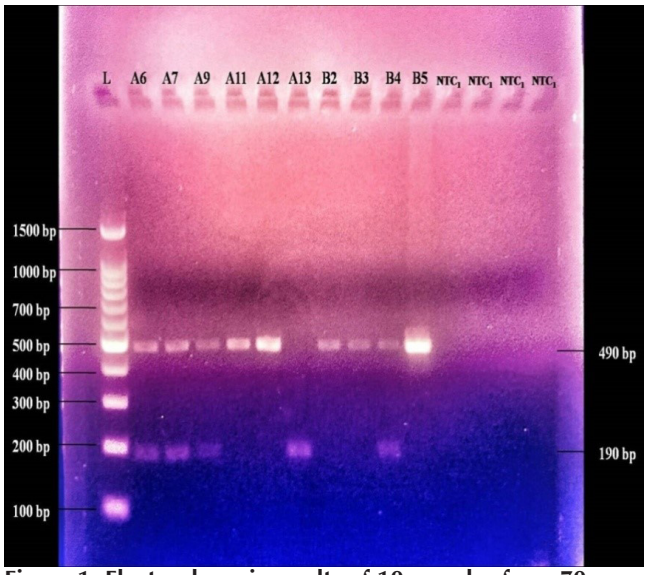


**Figure 1: Electrophoresis results of 10 samples from 70 samples amplified.** Samples with codes A6, A7, A9, A11, A12, A13, B2, B3, B4 and B5, the letter L is Ladder, and N1-N4 is None Template Control.

$\alpha > 0.05$, meaning that the sample population used in this study was not in Hardy-Weinberg legal equilibrium (p-value 0.854). Table IV shows that all genotypes (II, ID + DD) deviated from the Hardy-Weinberg equation.

Our results also showed a family history of hypertension as a risk factor for the incidence of hypertension. As many as 42 subjects of this study have a family history of hypertension, among which, 29 subjects (41.4%) were suffering from hypertension, and 13 subjects (18.6%) were normotensive. Furthermore, 28 subjects without a family history of hypertension recorded. Among these subjects, 6 (8.6%) were hypertensive, while the rest (22 subjects; 31.4%) had no hypertension. The Odds Ratio value of 8.179 supported the results of this study (Table III).

There were 28 people (40%) with a family history of ID + DD genotypes and 4 people (5.7%) without history (Table IV). The II genotypes were 14 people (20%) with a history and 24 people (34.3%) without history. The Odds Ratio value found to be 12.000, indicating that a family history of hypertension was a risk factor for ID + DD genotypes. The ACE I/D gene polymorphism was not a risk factor for hypertension (OR = 0.749). The study found no significant relationship between ACE I/D

**Table II: Genotype and allele frequencies of the ACE I/D gene in the Hypertensive and Normotensive groups**

| ACE I/D genes | Total | | *P-Value* |
|---|---|---|---|
| | **Hypertensive n (%)** | **Normotensive n (%)** | |
| Genotypes | | | |
| II | 20 (57.1) | 18 (51.4) | P ≠ 1 |
| ID | 12 (34.3) | 13 (37.1) | P ≠ 1 |
| DD | 3 (8.6) | 4 (11.5) | P ≠ 1 |
| **Total** | **35** | **35** | |
| Alleles | | | |
| I | 49 (70) | 52 (74.3) | |
| D | 21 (30) | 18 (25.7) | |
| **Total** | **70** | **70** | |

**Table III: Association of family history of hypertension and hypertension**

| Family History of Hypertension | Groups | | | | Total | | *P-value | OR |
|---|---|---|---|---|---|---|---|---|
| | Hypertensive | | Normotensive | | | | | |
| | N | % | n | % | n | % | | |
| Positive | 29 | 82.9 | 13 | 37.1 | 42 | 60.0 | 0.001 | 8.179 (2.683–24.939) |
| Negative | 6 | 17.1 | 22 | 62.9 | 28 | 40.0 | | |
| **Total** | **35** | **100.0** | **35** | **100.0** | **70** | **100.0** | | |

* Statistically significant ($p < 0.05$).

**Table IV: Association of family history of hypertension and ACE I/D genes polymorphism**

| Family History of Hypertension | ACE I/D Genes Polymorphism | | | | Total | | *P-value* | OR (lower – upper) |
|---|---|---|---|---|---|---|---|---|
| | ID + DD | | II | | | | | |
| | N | % | n | % | n | % | | |
| Positive | 28 | 87.5 | 14 | 36.8 | 42 | 60.0 | 0.001 | 12.000 (3.480 – 41.374) |
| Negative | 4 | 12.5 | 24 | 63.2 | 28 | 40.0 | | |
| **Total** | **32** | **100.0** | **38** | **100.0** | **70** | **100.0** | | |

* Statistically significant ($p < 0.05$).

**Table V: Association of ACE I/D genes polymorphism and hypertension**

| ACE I/D Genes Polymorphism | Groups | | | | Total | | *P-value* | OR (lower – upper) |
|---|---|---|---|---|---|---|---|---|
| | Hypertensive | | Normotensive | | | | | |
| | n | % | n | % | n | % | | |
| ID + DD | 15 | 42.9 | 17 | 48.6 | 32 | 45.7 | 0.631 | 0.749 (0.310-2.037) |
| II | 20 | 57.1 | 18 | 51.4 | 38 | 54.3 | | |
| **Total** | **35** | **100.0** | **35** | **100.0** | **70** | **100.0** | | |

* Statistically significant ($p < 0.05$).

gene polymorphism and the incidence of hypertension (p-value 0.634) (Table V).

## DISCUSSION

The Odds Ratio of family history of hypertension with the incidence of hypertension showed a value of 8.179, showing their high chances to have hypertension compared to subjects without history. In this study, 29 subjects with a family history of hypertension were hypertension-diagnosed and 13 subjects also with history were undiagnosed. As many as 13 subjects were recorded to have a family history with one hypertensive parent, among which, 7 subjects were normotensive. This indicated that the dominant allele of hypertension is not inherited among these subjects. On the other hand, the case of 6 other subjects with both hypertensive parents has proved hypertension as the silent killer, which often asymptomatic. These subjects were in the phase of prehypertension and usually undiagnosed during blood pressure checks. In addition, individuals with both hypertensive parents often show high diastolic blood pressure (6).

This study proved that the family history of hypertension had an important role as a determinant factor of subjects to suffer from hypertension. If left naturally without any prevention or treatment, the subject can suffer from hypertension with signs and symptoms. Our study also found a relationship between family history of hypertension with ACE I/D gene polymorphism. The OR value of this relationship was 12.000, showing that subject with a family history of hypertension tends to have 12.000 times risk for suffering from hypertension. A total of 28 subjects with a family history of hypertension with ID + DD allele were 23 subjects with ID genotype and 5 subjects with DD genotype. There were 14 subjects with a family history of hypertension with II alleles. Similarly, in line with theory, there are approximately 20-50 genes that regulate blood pressure. There are also a relationship between a family history of hypertension and parasympathetic nerve activity.

The incidence of hypertension caused not only by heredity but also influenced by lifestyle factors. These factors include high intake of fat and sodium as well as a lack of physical activity (20–23). Our study found four subjects without a family history of hypertension but was suffering from hypertension. The genotypic analysis showed ID + DD alleles by these subjects. Two subjects had ID genotype, and the others had DD genotype. Our results revealed that the polymorphism of ACE I/D gene polymorphism was not associated with the incidence of hypertension, in agreement with Rasyid et al (24). The study also found a significant relationship between ACE gene I/D polymorphism and pulse pressure. On the contrary, Abdel hamid (25) reported polymorphism as a molecular marker for hypertension due to a strong correlation between the ACE I/D gene polymorphism and an increased risk of hypertension and its complications. Steassen et al (26) showed a significant relationship between D allele and hypertension in Asian women and populations, while there was no association with other groups.

Based on our results, the polymorphism of ACE I/D gene was a protective variable of the incidence of hypertension in coastal communities of Kendari City. The mechanism protective factors of ACE I/D gene polymorphisms in the coastal population of Kendari City is still unknown. The distribution of the ACE genotype differs between ethnic groups throughout the world. Hardy-Weinberg equilibrium occurs if the population does not undergo evolution where allele and genotype frequencies in gene collections have not changed for several generations. This study described the genotypic distribution were not at Hardy-Weinberg equilibrium. All genotypes (II, ID, DD) in the population found to deviate from the equilibrium Hardy-Weinberg ($P \neq 1$).

## CONCLUSION

There was no significant correlation between ACE I/D gene polymorphism and the incidence of hypertension. Family history of hypertension was a risk factor for hypertension and significantly associated with ACE I/D gene polymorphism.

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