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ACE Gene Polymorphism in Children with Nephrotic Syndrome in the Indonesian Population

TEGUH HARYO SASONGKO[1], AHMAD HAMIM SADEWA[1], PUNGKY ARDANI KUSUMA[2], MARTUA PARLINDUNGAN DAMANIK[2], MYEONG JIN LEE[1], HITOSHI AYAKI[1], KANDAI NOZU[3], AKINOBU GOTO[3], MASAFUMI MATSUO[3], and HISAHIDE NISHIO[1]

[1] *Division of Public Health, Kobe University Graduate School of Medicine*
[2] *Gadjah Mada University School of Medicine, Pediatrics, Yogyakarta, DIY, Indonesia*
[3] *International Center for Medical Research and Treatment, Kobe University School of Medicine, Kobe, Hyogo, Japan*

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Background: The angiotensin converting enzyme (ACE) gene carries insertion (I) and deletion (D) polymorphism within its intron 16. The presence of D-allele in the ACE gene has been reported as a probable genetic risk factor for idiopathic nephrotic syndrome (INS), especially the subtype of focal segmental glomerulosclerosis (FSGS). The D-allele may be related to poor responsiveness to steroid therapy. To clarify the relationship between the D-allele and INS, we studied the prevalence of the D-allele in the Javanese-Indonesian patients. Additionally, we also analyzed relationship between each genotype and steroid sensitivity among the MCNS patients.

Methods: Eighty-five Javanese-Indonesian patients under 15 years of age with INS were enrolled in this study: 16 patients with FSGS and 69 patients with minimal change nephrotic syndrome (MCNS). As controls, 68 healthy adult Javanese-Indonesians with no history of kidney disease volunteered to participate in this study. Genotypes based on the polymorphisms (I/D) were determined by using a PCR method. As for the steroid responsiveness, the information of 14 out of 16 FSGS patient (87.5%) and 69 out of 69 MCNS patients (100%) was available.

Results: The genotype frequencies in the FSGS patients were II 37% (6/16), ID 44% (7/16) and DD 19% (3/16), and the D-allele frequency was 41% (13/32). The genotype frequencies in the MCNS patients were II 56% (39/69), ID 38% (26/69) and DD 6% (4/69), and the D-allele frequency was 25% (34/138). The genotype frequencies in the controls were II 60% (41/68), ID 31% (21/68), and DD 9% (6/68), and the D-allele frequency was 26% (33/136). None of the FSGS patients were sensitive to steroid, while almost all MCNS patients (66/69) were sensitive to steroid. The genotype frequencies among steroid-sensitive MCNS patients were consistent with those of the controls, suggesting that there was no relationship between each genotype and steroid sensitivity.

Conclusions: In the Javanese-Indonesian population, none of the comparisons showed any significant differences in the genotypic distribution and allelic frequencies among the three groups, FSGS, MCNS and controls, although D-allele tended to exist more frequently in FSGS patients than in the MCNS patients and controls. In addition, the D-allele frequency was not related to steroid sensitivity in the MCNS patients.

Phone: +81-78-382-5542 Fax: +81-78-382-5559 Email : teguhharyosasongko@yahoo.com

INTRODUCTION

Angiotensin converting enzyme (ACE) is a key enzyme that converts inactive angiotensin I into a vasoactive and aldosterone-stimulating peptide angiotensin II. In some cases, the increase of ACE protein is responsible for the elevation of angiotensin II level. Elevated angiotensin II level makes deleterious effects on renal hemodynamics and induces the expression of other growth factors, leading to glomerulosclerosis (7).

The ACE gene carries insertion (I) and deletion (D) polymorphism, and the DD-genotype is reportedly related to an increase in the ACE protein expression (9). Therefore, it has been thought that the DD genotype may link to the ACE-related pathophysiology of renal diseases (4,6). Of the ACE I/D polymorphism impacts on the renal diseases, idiopathic nephrotic syndrome (INS) holds particular attention, especially the focal segmental glomerulosclerosis (FSGS). Hori et al. (4) reported that the frequency of DD genotype was higher in FSGS patients than in controls. Lee et al. (6) reported that FSGS patients with DD genotype showed a lower responsiveness to corticosteroid therapy and a higher incidence of chronic renal failure than those with other genotypes.

Although there are many reports from other populations, there is no studies on the relationships between ACE I/D polymorphism and renal diseases have been reported from the Indonesian population. Here, we determined the distribution of the ACE I/D polymorphism among INS patients and healthy individuals in the Javanese-Indonesian population, and compared our results with the data reported from other populations.

SUBJECTS AND METHODS

Subjects

A total of 85 Javanese-Indonesian patients with INS who visited Sardjito General Hospital in Yogyakarta, Indonesia, were enrolled in this study: 16 patients with FSGS (male/female: 9/7, age: 1.5~15) and 69 patients with minimal change nephrotic syndrome (MCNS) (male/female: 38/31 age: 1.6~13). Javanese-Indonesian is defined as native inhabitants of Java Island. FSGS and MCNS were diagnosed based on renal biopsy findings. As controls, 68 non-related healthy individuals living in Java Island volunteered to participate in this study. DNA analysis was performed after obtaining informed consent from the patients and/or the patient's parents. The ethical committee, Gadjah Mada University School of Medicine, approved this study plan**.**

Among the 16 FSGS patients, 10 were dependent on steroid and 4 resistant to steroid. The other two patients were omitted from the analysis for steroid responsiveness, because their information was not available. Among the 69 MCNS patients, 66 were sensitive to steroid and 3 dependent on steroid. Steroid sensitive condition is defined as excreting protein-free urine (less than 100 mg/m^2 per day) for at least 3 consecutive days during 8 weeks of initial therapy (6,7). Meanwhile, steroid dependent condition is defined as the tendency to relapse while on or during tapering of steroid dose, or within 14 days of steroid withdrawal. In this study, steroid-responsive patients included steroid-sensitive and steroid-dependent patients. Steroid resistant condition is defined as excreting urinary protein exceeding 1,000 mg/m^2 per day without any clinical improvement (7).

ACE Gene Insertion/Deletion Polymorphism Genotyping

Genomic DNA was extracted from whole blood using SepaGene Kit (Sanyo Junyaku Co., Ltd., Tokyo, Japan). PCR was carried out according to the method of Rigat B et al (8). The sequences of the forward and reverse primers were: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3', respectively (8).

Statistics

The genotype and allele frequencies (II, ID and DD-genotypes, I- and D-alleles) with 95% confidence intervals (95% CI) were calculated using the normal approximation. Exact confidence intervals were used when observed frequencies were zero. The genotype and allele frequencies were also compared using a chi-square test. A *p* value below 0.05 was considered to be significant.

RESULTS

Figure 1 presents the electrophoresis patterns of PCR products from the three genotypes. Individuals with II-genotype showed a single intense band of 490 bp, those with ID-genotype showed two intense bands of 490 bp and 190 bp, and those with DD-genotype showed a single intense band of 190 bp.

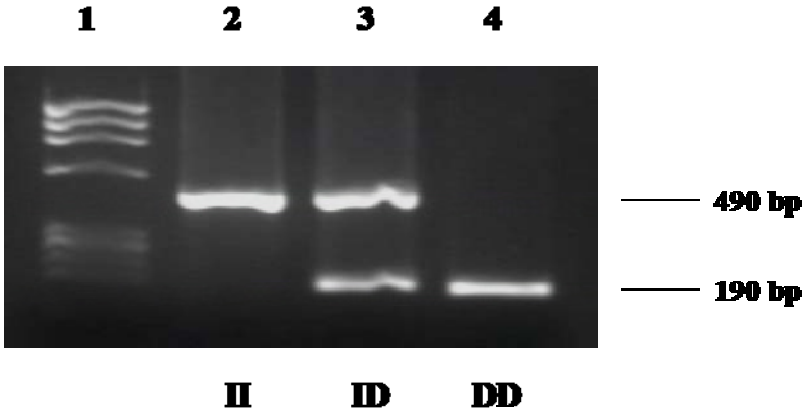

Figure 1. Insertion/deletion (I/D) polymorphisms of the ACE gene.
The amplification product was electrophoresed on 3% agarose gel and visualized by ethidium bromide staining. Lane 1: marker of PhiX174 DNA-Hae III Digest, Lane 2: II-genotype, Lane 3: ID-genotype, Lane 4: DD-genotype.

The genotype frequencies in the FSGS patients were II 37% (6/16), ID 44% (7/16) and DD 19% (3/16), and the D-allele frequency was 41% (13/32). The genotype frequencies in the MCNS patients were II 56% (39/69), ID 38% (26/69) and DD 6% (4/69), and the D-allele frequency was 25% (34/138). The genotype frequencies in the controls were II 60% (41/68), ID 31% (21/68), and DD 9% (6/68), and the D-allele frequency was 26% (33/136).

Table 1 shows the frequencies of genotypes and alleles in two INS groups, FSGS and MCNS, and one control group in the Javanese-Indonesian population. A comparison using chi-square test showed no significant differences in the D-allele frequency between the FSGS patients and controls, between the MCNS patients and controls, or between the FSGS patients and MCNS patients. However, it is noteworthy that D-allele tended to exist more frequently in the FSGS patients than in MCNS patients and controls, although statistically not significant.

Table 1. Prevalence of ACE I and D polymorphism genotypes and alleles in Javanese Indonesian Idiopathic Nephrotic Syndrome patients

	FSGS (%)	MCNS (%)	Control (%)	*p* Value[1]		
	n = 16	n = 69	n = 68	FSGS vs Cont	FSGS vs MCNS	MCNS vs Cont
II	6 (37)	39 (56)	41 (60)	0.219	0.161	0.614
ID	7 (44)	26 (38)	21 (31)			
DD	3 (19)	4 (6)	6 (9)			
I-allele	19 (59)	104 (75)	103 (76)	0.062	0.068	0.943
D-allele	13 (41)	34 (25)	33 (24)			

[1] *p* Value in chi-square test for the comparison of two populations

The genotype distributions and the allele frequencies in FSGS patients reported from various ethnic populations are summarized in Table 2. Although the majority of previous studies showed no significant relationship between D allele and FSGS, data from Japan and Korea indicated significant relationship between D allele and FSGS incidence and/or disease severity.

Ten of the 14 FSGS patients whose information on steroid responsiveness was available were resistant to steroid, and 4 of them were dependent on steroid. None of the 14 FSGS patients were sensitive to steroid, which means that steroid therapy did not bring complete remission to them. On the contrary, almost all of MCNS patients (66/69) were sensitive to steroid. Therefore, MCNS patients are significantly more sensitive to steroid than FSGS patients.

The distributions of ACE genotypes among the patients are summarized according to their responsiveness to the steroid therapy (Table 3). To clarify the relationship between the steroid responsiveness and each genotype, we analyzed the MCNS patients sensitive to the steroid therapy. The genotype frequencies in the steroid-sensitive MCNS patients were consistent with those of the controls, suggesting that there was no relationship between each genotype and steroid sensitivity in MCNS.

Table 2. Prevalence of genotypic and allelic ACE I/D polymorphism in FSGS patients of different populations

Population (Reference)	Genotype/ Allele	Control (%)	FSGS (%)	*p* Value FSGS vs Control
Javanese Indonesian (This Study)		(n = 68)	(n = 16)	
	II	41 (60)	6 (37)	0.219
	ID	21 (31)	7 (44)	
	DD	6 (9)	3 (19)	
	I	103 (76)	19 (59)	0.062
	D	33 (24)	13 (41)	
Korean [7]		(n = 61)	(n = 30)	
	II	27 (44)	4 (13)	0.003*
	ID	25 (41)	14 (47)	
	DD	9 (15)	12 (40)	
	I	79 (65)	22 (37)	0.0003*
	D	43 (35)	38 (63)	
Kuwaiti Arab [1]		(n = 48)	(n = 3)	
	II	1 (2)	0 (0)	0.27
	ID	22 (46)	0 (0)	
	DD	25 (52)	3 (100)	
	I	24 (25)	0 (0)	0.161
	D	72 (75)	6 (100)	
Israeli [4]		(n = 216)	(n = 47)	
	II	22 (10)	6 (13)	0.573
	ID	110 (51)	20 (42)	
	DD	84 (39)	21 (45)	
	I	154 (36)	32 (34)	0.768
	D	278 (64)	62 (66)	
African-American [3]		(n = 18)	(n = 21)	
	II	0 (0)	3 (14)	0.233
	ID	13 (72)	12 (57)	
	DD	5 (28)	6 (29)	
	I	13 (36)	18 (43)	0.544
	D	23 (64)	24 (57)	
White/Hispanic [3]		(n = 22)	(n = 26)	
	II	3 (14)	9 (35)	0.069
	ID	11 (50)	14 (54)	
	DD	8 (36)	3 (11)	
	I	17 (39)	32 (62)	0.025**
	D	27 (61)	20 (38)	
Turkish [8]		(n = 76)	(n = 30)	
	II	10 (13)	3 (10)	0.332
	ID	53 (70)	18 (60)	
	DD	13 (17)	9 (30)	
	I	73 (48)	24 (40)	0.291
	D	79 (52)	36 (60)	
Japanese [5]		(n = 130)	(n = 43)	
	I	174 (67)	45 (52)	0.015*
	D	86 (33)	41 (48)	

* Significant

**The paper concluded that there is no significant difference between the two groups

Table 3. Distribution of ACE Polymorphisms among Steroid Responsiveness Groups

	MCNS			FSGS		
	S	D	R	S	D	R
II	37	2	0	0	2	4
ID	25	1	0	0	1	4
DD	4	0	0	0	1	2

S = Sensitive; D = Dependent; R = Resistant

DISCUSSION

This is the first report of ACE I/D polymorphism in the Javanese-Indonesian population, as far as we know. Highlights on the use of ACE inhibitor for FSGS patients give rise to the curiosity of ACE I/D polymorphism involvement in the course of FSGS. In fact, ACE antagonist administration has been reportedly beneficial to FSGS patients in decreasing urinary protein excretion and preventing progression to renal failure (5). A previous study showed that ACE DD genotype in FSGS revealed to be a possible risk factor for the poor responsiveness to steroid (6).

In our study, there was no significant relationship between D allele frequency and INS, although D allele frequency was apt to be higher in FSGS patients than in MCNS patients and controls (Table 1). The reports from Korea (6) and Japan (4) stated that the D allele frequency was significantly higher in FSGS patients than in controls. The Korean report also indicated significant relationship between D allele and clinical severity. Several other studies did not observe any relationship between D allele and FSGS (1,2,3,7) (Table 2). In White/Hispanic population (2), D allele tends to exist less frequently in the FSGS patients than in controls. Contribution of the gene polymorphism to the incidence or clinical severity of FSGS varies among ethnicities.

Our study revealed that while almost all MCNS patients were sensitive to steroid, none of the FSGS patients showed such sensitivity ($p < 0.05$). These data, in accordance with previous reports, indicated that patients with MCNS respond much better to the steroid therapy than those with FSGS do. However, we could not completely neglect the steroid therapy for FSGS patients. Four of the 14 FSGS patients with available information were dependent on steroid, suggesting that steroid has some effect on the decrease in urinary protein excretion.

In our study, we also examined the relationship between genotypes and steroid sensitivity of the MCNS patients. We found that the frequencies of each genotype in steroid-sensitive MCNS patients were consistent with those of the controls. This suggested that those genotypes are not related to steroid responsiveness status in MCNS.

In conclusion, in the Javanese-Indonesian population, none of the comparisons among the three groups, FSGS, MCNS and controls, showed any significant differences in the genotypic distribution and allelic frequencies, although D-allele tended to exist more frequently in FSGS patients than in healthy individuals and MCNS patients. While we revealed that patients with MCNS respond much better to steroid compared to those with FSGS, each genotype was not related to the sensitivity to steroid in the MCNS patients. To investigate the exact relations of the D allele frequency and FSGS, it would certainly be worthwhile to examine larger number of patients and controls, taking account of ethnicity.

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