RESEARCH ARTICLE

Effects of *ACE* rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T Polymorphisms on Angiotensin-Converting Enzyme Activity among Thai Subjects

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Received: 31 October 2019; **Revised:** 9 December 2019
Accepted: 25 December 2019

Abstract

Angiotensin-converting enzyme (ACE) metabolizes bradykinin and substance P. Variation in *ACE* gene alters ACE activity, consequently increasing or decreasing the bradykinin and substance P levels in the respiratory tract. This might explain the differences in incidence of cough due to ACE inhibitors according to ethnicity. This study aimed to investigate the influence of *ACE* polymorphisms (rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T) on serum ACE activity in Thai population. Allele frequencies of the SNPs from this study and those previously reported in other populations were also compared. Real-time polymerase chain reaction-based assay was used to detect *ACE* SNPs in genomic DNA. ACE activity was measured by a spectrophotometer. Among 100 Thai healthy volunteers, the frequencies of variant alleles for rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T were 0.28, 0.28, 0.29 and 0.33, respectively. The results showed significant differences in variant allele frequencies of rs4311C>T, rs4343A>G and rs4344A>G between Thai and other populations ($p<0.05$). Significant increases in median ACE activity were observed in subjects with homozygous variant alleles of the following SNPs compared to those with homozygous wild-type allele: rs4311C>T (52 *vs.* 26 U/L, $p=0.001$), rs4343A>G (52 *vs.* 26 U/L, $p=0.000$), rs4344A>G (52 *vs.* 26 U/L, $p=0.003$) and rs4362C>T (48 *vs.* 24 U/L, $p=0.001$). In conclusion, our study demonstrated the effects of *ACE* (rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T) polymorphisms on ACE activity in Thai subjects. Further studies should be performed to elucidate the relationships between these *ACE* polymorphisms and incidence of cough related to ACE inhibitors in Thai population.

Keywords: Angiotensin-converting enzyme, ACE, SNP, polymorphism

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ผลของภาวะพหุสัณฐานของยีน *ACE* rs4311C>T, rs4343A>G, rs4344A>G และ rs4362C>T ต่อการทำงานของแองจิโอเทนซิน-คอนเวอร์ติงเอนไซม์ในชาวไทย

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รับบทความ: 31 ตุลาคม 2562; **แก้ไข:** 9 ธันวาคม 2562
ตอบรับ: 25 ธันวาคม 2562

บทคัดย่อ

แองจิโอเทนซินคอนเวอร์ติงเอนไซม์ (ACE) มีหน้าที่เปลี่ยนแปลง bradykinin และ substance P ความแปรปรวนของยีน *ACE* ทำให้การทำงานของ ACE เปลี่ยนแปลงไป ส่งผลทำให้ระดับ bradykinin และ substance P ในระบบทางเดินหายใจเพิ่มขึ้นหรือลดลง ซึ่งอาจอธิบายความแตกต่างทางเชื้อชาติของอุบัติการณ์ของการไอที่เกิดจากยายับยั้ง ACE การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของภาวะพหุสัณฐานของยีน *ACE* (rs4311C>T, rs4343A>G, rs4344A>G และ rs4362C>T) ต่อการทำงานของ ACE ในซีรัมในกลุ่มประชากรไทย และเปรียบเทียบความถี่ของ variant allele ของ SNPs ดังกล่าวกับกลุ่มประชากรอื่นที่เคยมีรายงานก่อนหน้านี้ การวิเคราะห์ *ACE* SNPs ในดีเอ็นเอจากจีโนมอาศัย real-time polymerase chain reaction based assay และวัดการทำงานของ ACE โดยสเปกโทรโฟโตมิเตอร์ ความถี่ของ variant allele สำหรับ rs4311C>T, rs4343A>G, rs4344A>G และ rs4362C>T ในอาสาสมัครสุขภาพดีชาวไทยจำนวน 100 คน เท่ากับ 0.28, 0.28, 0.29 และ 0.33 ตามลำดับ ผลการศึกษาพบความแตกต่างอย่างมีนัยสำคัญระหว่างความถี่ของ variant allele สำหรับ rs4311C>T, rs4343A>G และ rs4344A>G ในกลุ่มประชากรไทยกับกลุ่มประชากรอื่น (p<0.05) ผู้ที่มี homozygous variant alleles ของ SNPs ต่อไปนี้มีค่ามัธยฐานของการทำงานของ ACE สูงกว่าผู้ที่มี homozygous wild-type allele อย่างมีนัยสำคัญ: rs4311C>T (52 และ 26 U/L, p=0.001), rs4343A>G (52 และ26 U/L, p=0.000), rs4344A>G (52 และ 26 U/L, p=0.003) และ rs4362C>T (48 และ 24 U/L, p=0.001) โดยสรุป การศึกษานี้แสดงให้เห็นผลของภาวะพหุสัณฐานของยีน *ACE* (rs4311C>T, rs4343A>G, rs4344A>G และ rs4362C>T) ต่อการทำงานของ ACE ในชาวไทย ดังนั้น ควรมีการศึกษาความสัมพันธ์ระหว่างภาวะพหุสัณฐานของยีน *ACE* กับอุบัติการณ์ของการไอเนื่องจากยายับยั้ง ACE ในกลุ่มประชากรไทยต่อไป

คำสำคัญ: แองจิโอเทนซินคอนเวอร์ติงเอนไซม์, ACE, SNP, ภาวะพหุสัณฐาน

Introduction

Angiotensin-converting enzyme (ACE) inhibitors have a major role in the treatment of cardiovascular diseases and renal impairment.[1] ACE inhibitors, but not angiotensin receptor blockers (ARBs) reduce cardiovascular death and prevent myocardial infarction in hypertensive patients.[2] Moreover, the risk of renal failure in patients with chronic kidney disease is lower with ACE inhibitors than with ARBs.[3] However, one of the limitation of continuing ACE inhibitors is their adverse reactions, especially dry cough.[4] The incidence of cough leading to discontinuation of ACE inhibitors varied among ethnicity, e.g., 9.6% in Africans[5], 30.4% in Singaporeans[6] and 47% in Chinese.[7] In Thailand, the incidence of cough induced by ACE inhibitors was 23.6% from a retrospective study and 31.3% from a prospective study.[8]

ACE is recognized as peptidyl-dipeptidase A or kininase II (EC 3.4.15.1) and has important role in bradykinin and substance P metabolism. Inhibition of ACE by ACE inhibitors results in increases in bradykinin and substance P levels, which might be a mechanism of ACE inhibitor-induced cough.[9,10] ACE is encoded by *ACE* gene, located on chromosome 17q23.[11] In the National Center for Biotechnology Information (NCBI) database, there are nine variant types of *ACE* gene in *Homo sapiens*, i.e., single nucleotide polymorphism (SNP), copy number variation, insertion, deletion, short tandem repeat variation, inversion, mobile element insertion, sequence alteration and tandem duplication.[12] Currently, 7,246 SNPs have been observed in *ACE* gene.[12]

Several studies have reported the influence of *ACE* polymorphisms on ACE activity, bradykinin metabolite level, and the incidence of ACE inhibitor-induced cough in various ethnic groups. For *ACE* insertion(I)/deletion(D) polymorphisms, subjects with *ACE* D/D genotype had significantly higher plasma ACE activity and level of BK1-5, the inactive metabolite of bradykinin, compared to I/I homozygous subjects.[13,14] A study by the Yamagata University Genomic Cohort Consortium (YUGCC) have shown associations of rs4311C>T SNP in the *ACE* gene with plasma levels of ACE in Japanese populations in Takahata ($p=2.59\times10^{-62}$) and Yamagata ($p=1.44\times10^{-49}$).[15] In this study, plasma level of ACE was also affected by *ACE* rs4343A>G SNP in Takahata residents ($p=1.14\times10^{-54}$) and Yamagata residents ($p=9.92\times10^{-63}$).[15] In addition, rs4343A>G SNP significantly influenced the serum ACE activity ($p=3.00\times10^{-25}$) in Han Chinese with young-onset hypertension.[16] In patients with GG genotype for rs4343A>G, two polymorphisms (rs4459610 and rs4267385) in the *ACE* gene were found to be protective against cough due to ACE inhibitors.[17] The rs4344A>G SNP increased the risk of cough by ACE inhibitors in female Spanish patients ($p=0.031$, OR=1.847, 95% CI:1.055-3.234) but had protective effect in males ($p=0.027$, OR= 0.560, 95% CI: 0.334-0.938).[18] Strong associations of rs4362C>T polymorphism with plasma ACE activity ($p=1.55\times10^{-164}$)[19] and with ACE level ($p=3.44\times10^{-104}$) in Japanese population were demonstrated.[15]

Up to now, there has been no study examining the impact of rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T SNPs in the *ACE* gene on serum ACE activity in Thai population. The purpose of this study was to assess the effects of *ACE* polymorphisms (rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T) on ACE activity in serum among Thai subjects. Additionally, the allele frequencies of these SNPs in Thais were compared with those of other populations.

Materials and Methods

Study subjects

This study was approved by the Human Research Ethics Committee of Thammasat University No.1 (Faculty of Medicine), Thailand (COA no. 045/2561). Healthy subjects aged more than 18 years were included in the study. The exclusion criteria were pregnancy, breastfeeding, smoking, alcohol or drug abuse, and medical problems (diabetes mellitus, hypertension, heart disease, thyroid disease, cirrhosis, leprosy, cancer and AIDS). Subjects taking herbal or steroidal medicines were advised to stop at least 2 weeks before entering this study.

Blood sample collection

Blood samples from healthy volunteers were collected from veins and then incubated at room temperature for 1 h. Serum was separated by centrifugation at 3,500 rpm at 4℃ for 10 min and stored at -20℃ for further analysis of ACE activity. The whole blood samples were kept at 2-8℃ for DNA isolation.

Determination of serum ACE activity

ACE activity in serum was determined according to the method by Ronca-Testoni et al.[20] The substrate furanacryloyl-L-phenylalanylglycylglycine (FAPGG) (Sigma-Aldrich, St. Louis, MO, USA) is hydrolyzed by ACE into furyl-acryloyl-L-phenylalanine and glycylglycine. In brief, a serum sample (50 µL) was added to 500 µL of substrate-buffer solution (containing FAPGG 0.8 mM, NaCl 0.3 M and borate 80 mM, pH 8.2) and distilled water (450 µL). The mixture was incubated at 37℃ for 10 min and then the absorbance was measured at 345 nm at time interval of 10 min using a UV-VIS spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The assay was performed in duplicate. ACE activity was calculated by the following equation: ACE activity (U/L) = $(\Delta A/\text{min} \times V_t \times 1000)/(0.5 \times V_s)$, where ΔA means changes in absorbance, V_t is the final assay volume (1 mL) and V_s is serum volume (50 µL).

DNA isolation

The extraction of genomic DNA from whole blood was performed using QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). A NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) was used to quantify concentration and evaluate purity of DNA. The isolated DNA samples were normalized to a concentration of 20 ng/µL with nuclease-free water and was stored at -20℃.

Detection of ACE polymorphisms

Genotyping of *ACE* rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T SNPs was performed by using TaqMan™ SNP genotyping assays (assay IDs: C___1247707_1_, C__11942562_20, C__11942565_10 and C___1247717_1_, respectively). The real-time polymerase chain reaction (PCR) (Applied Biosystems, Foster city, CA, USA) was used to genotype DNA samples for *ACE* SNPs according to TaqMan® genotyping master mix protocol. The genotyping was carried out in duplicate. The real-time PCR process involved initial enzyme activation at 95℃ for 10 min, followed by 40 cycles consisting of a denaturation step at 95℃ for 15 s, annealing and extension at 60℃ for 60 s.

Statistical analysis

The observed and expected genotype frequencies were tested for Hardy-Weinberg equilibrium using Chi-squared test. The differences in allele frequencies between Thai healthy subjects and those previously reported in other populations were assessed by Fisher's exact test. To test non-normal distribution of data, Shapiro-Wilk and Kolmogorov-Smirnov tests were used. Percentage, median and inter-quartile range (IQR) were used to describe data. The relationship between age and serum ACE activity was determined by Spearman's rank correlation. The serum ACE activities in different genotypes were compared using Mann-Whitney U test. A significant difference was considered if *p*-values were less than 0.05.

Results

The demographics and baseline characteristics of the study subjects are summarized in Table 1. A total of 100 healthy Thai volunteers fulfilled the eligibility criteria. Seventy-five subjects (75%) were female. The median age was 20 (range 19-25) and the median serum ACE activity was 30 (range 10-88) U/L. A significantly higher ACE activity (162%, p=0.010) was observed in males compared to females (median: 44 *vs.* 26, IQR: 22-42 *vs.* 30-48 U/L) (Table 2). There was no correlation between serum ACE activity and age (rho=0.059, p=0.560).

The genotype frequencies of *ACE* rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T SNPs are shown in Table 3. For ACE rs4311C>T, 51 subjects were homozygous wild-type and 7 subjects were homozygous variants. Regarding *ACE* rs4343A>G, 50 subjects with AA genotype and 7 subjects with GG genotype were found. For *ACE* rs4344A>G, there were 49 subjects with homozygous variants and 7 subjects with homozygous wild-type. For *ACE* rs4362C>T SNP, CC genotype and TT genotype were observed in 43 and 9 subjects, respectively. The observed genotype frequencies of all SNPs in this study were in Hardy-Weinberg equilibrium with no statistically significant differences from the expected genotype frequencies.

Table 1. Summary of demographics and baseline information of study subjects.

	Median (range) (N=100)
Female	75 (75%)[a]
Age (years)	20 (19-25)
ACE activity in serum (U/L)	30 (10-88)

[a]Data are given as number (%)

Table 2. Influence of gender and serum ACE activity.

Gender	**N**	**Serum ACE activity (U/L)**	
		Median	**IQR**
Male	75	44	30-48
Female	25	26	22-42

Table 3. Genotype frequencies of *ACE* rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T in Thai healthy volunteers (N = 100).

Genotype	Genotype frequency (%)		$\chi 2$	*P*-value
	Observed	Expected		
***ACE* rs4311C>T**			0.174	0.677
CC	51	52		
CT	42	40		
TT	7	8		
***ACE* rs4343A>G**			0.303	0.582
AA	50	50		
AG	43	43		
GG	7	7		
***ACE* rs4344A>G**			0.469	0.493
AA	49	50		
AG	44	41		
GG	7	9		
***ACE* rs4362C>T**			0.731	0.393
CC	43	45		
CT	48	44		
TT	9	11		

The comparison of variant allele frequencies for *ACE* rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T between Thais and previously reported populations (Asians, Caucasians, Africans, Hispanic or Latino) is summarized in Table 4.

Thais had a significantly lower prevalence (1.8-fold) of *ACE* rs4311 T allele (0.28) compared to Caucasians (0.50) (p=0.001).[21] However, significant differences in variant allele frequencies for *ACE* rs4311C>T SNP in Thais and previously reported in South Asians (0.32)[21] (p=0.537), East Asians (0.33)[21] (p=0.537) and Africans (0.21)[21] (p=0.250) were not observed. Our study showed that the variant allele for *ACE* rs4343A>G in the Thai population (0.28) was approximately 2-fold less frequent than that in Caucasians (0.57).[22] However, there was no significant difference in G allele frequency for this variant between Thais and other populations, i.e., African (0.20)[22], East Asian (0.32)[22], and South Asians[22] (0.37) (p=0.185, 0.537 and 0.174, respectively).

The G allele frequency for *ACE* rs4344A>G (0.29) was significantly lower (about 2-fold) in Thais compared to Caucasians (0.58)[23] (p=0.000) and Africans (0.60)[23] (p=0.000). However, no significant difference in G allele frequencies was observed between Thais and East Asians (0.33)[23] (p=0.605) as well as South Asians (0.38)[23] (p=0.178). The variant allele frequency for *ACE* rs4362C>T SNP in Thais (0.33) demonstrated in the present study was significantly lower than that in Caucasians (0.56)[24] (p=0.001). However, the allele frequencies of T alleles were not significantly different between Thais and those previously reported in South Asians (0.33)[24] (p=1.000), Africans (0.38)[24] (p=0.460) and East Asians (0.39)[24] (p=0.377).

Table 4. Comparison of variant allele frequencies of *ACE* rs4311C>T, rs4343A>G, rs4344A>G and rs4344A>G between Thai healthy volunteers and those previously reported in other populations.[21-24]

Populations / *ACE* SNPs	Variant allele frequencies				
	Asian			Caucasian	African
	Thai	East Asian	South Asian		
rs4311C>T	0.28	0.33	0.32	0.50*	0.21
rs4343A>G	0.28	0.32	0.37	0.57*	0.20
rs4344A>G	0.29	0.33	0.38	0.58*	0.60*
rs4362C>T	0.33	0.39	0.33	0.56*	0.38

*Statistical differences in allele frequencies between Thais and those previously reported in other populations, p value <0.05.

The influence of *ACE* rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T SNPs on serum ACE activity in Thai healthy volunteers is shown in Table 5. Subjects with TT genotype of *ACE* rs4311C>T SNP had significantly higher serum ACE activity compared to those with CC genotype (median: 52 *vs.* 26, IQR: 45-68 *vs.* 18-37 U/L) (p=0.001). Higher serum ACE activity was also observed in subjects with homozygous variant for *ACE* rs4343A>G compared to *ACE* rs4343A>G homozygotes (median: 52 *vs.* 26, IQR: 45-68 *vs.*18-34 U/L) (p=0.000). For *ACE* rs4344A>G polymorphism, subjects with *ACE* rs4344G homozygosity had higher serum ACE than in subjects with *ACE* rs4344A homozygosity (median: 52 *vs.* 26, IQR: 45-68 *vs.*18-32 U/L) (p=0.003). Moreover, the serum ACE activity of subjects with TT genotype of *ACE* rs4362C>T was significantly higher when compared to that of subjects with CC genotype (median: 48 *vs.* 24, IQR: 40-66 *vs.* 18-33 U/L) (p=0.002).

Discussion

Discontinuation of ACE inhibitors due to cough leading occurred in nearly 20% of patients.[10] Incidence of cough varies according to ethnicity.[6] Two studies at the out-patient department, Siriraj Hospital reported that the incidences of cough related to ACE inhibitors in Thai patients with hypertension were 23.6% and 31.3%.[8] Bradykinin and substance P are metabolized by ACE. Inhibition of ACE results in increased activity and concentration of these mediators. Accumulation of bradykinin and substance P in both upper and lower respiratory system by ACE inhibitor therapy precipitates the cough.[10] However, this proposed mechanism underlying cough due to ACE inhibitors remains controversial since it is not observed in all ACE inhibitor users.[10] We hypothesized that race-related *ACE* polymorphisms may influence ACE activity, consequently explain the variation in incidences of ACE inhibitor-induced cough across races.

Kawakami and colleagues[25] reported that the incidence of cough due to ACE inhibitor in subjects with D/D genotype of *ACE* I/D polymorphisms was lower than that in subjects with I/I genotype. They hypothesized that lower ACE levels in I/I genotype might relate to decreased metabolism of cough-inducing metabolites such as substance P and subsequently stimulating dry cough in patients who use

Table 5. Influence of *ACE* rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T SNPs on serum ACE activity in Thai healthy volunteers (N=100).

Genotype	Serum ACE activity (U/L)		
	Median (IQR)	Minimum	Maximum
***ACE* rs4311C>T**			
CC	26 (18-37)	12	70
CT	35 (24-48)	10	88
TT	52 (45-68)	40	74
***ACE* rs4343A>G**			
AA	26 (18-34)	12	70
AG	40 (26-49)	10	88
GG	52 (45-68)	40	74
***ACE* rs4344A>G**			
AA	26 (18-32)	12	70
AG	40 (26-49)	10	88
GG	52 (45-68)	40	74
***ACE* rs4362C>T**			
CC	24 (18-33)	12	66
CT	38 (26-51)	10	88
TT	48 (40-66)	26	74

ACE inhibitors. Interestingly, our study in Thai healthy subjects revealed that homozygous variant alleles of *ACE* rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T SNPs were correlated with higher ACE activity in serum when compared to homozygous wild-type allele. Since ACE (kininase II) is responsible for metabolizing substance P[26] and bradykinin to inactive metabolite (BK1-5)[27], subjects with *ACE* rs4311TT, rs4343GG, rs4344GG or rs4362TT genotype might have lower substance P and bradykinin concentration or activity in the respiratory system and have less frequent cough from ACE inhibition when compared to subjects with *ACE* rs4311CC, rs4343AA, rs4344AA or rs4362TT genotype.

Genome-wide association studies (GWS) in *ACE* gene have been performed in Japanese populations in Takahata and Yamagata. ACE levels in plasma were significantly associated with *ACE* rs4311C>T SNP.[15] Besides ACE levels, our study also investigated the impact of this SNP on serum ACE activity. Median serum ACE activity in TT genotype subjects of rs4311C>T SNP was significantly higher compared with CC genotype, which is consistent with those reported in Japanese populations. The variant allele frequency in Thai subjects (0.28) was significantly lower than that in Caucasian subjects. Therefore, regarding *ACE* rs4311C>T polymorphism, Thai subjects might be exposed to higher risk of cough-associated with ACE inhibitors than Caucasians.

The *ACE* rs4343A>G SNP was correlated with both ACE levels and ACE activity. The YUGCC study reported significant relationship between rs4343A>G SNP in *ACE* gene and serum ACE level in Japanese in Takahata and Yamagata.[15]

In addition, *ACE* rs4343A>G polymorphism in Han Chinese patients with young-onset hypertension exhibited a strong correlation with ACE activity in serum.[16] The mean serum ACE activity of patients with GG genotype was 18.75 U/L which was significantly higher than those with A/G (15.17 U/L) and AA genotypes (11.58 U/L). The direction of this relationship between *ACE* rs4343A>G SNP and serum ACE activity was similar to those observed in our study, i.e., the highest median serum ACE activity was found in Thai individuals with *ACE* rs4343GG while the lowest median serum ACE activity was noticed in those with AA genotype. Mas et al.[17] analyzed *ACE* rs4459610A>T and rs4267385C>T polymorphisms in Caucasian hypertensive patients with *ACE* rs4343G homozygosity who received ACE inhibitors and found that occurrence of ACE inhibitor-induced cough significantly decreased in patients with both GG genotype of rs4343 and homozygous variant allele of rs4459610 or rs4267385 compared to patients with rs4459610 AA +AT or rs426738 CC+CT genotypes. Therefore, the protective properties against ACE inhibitor-induced cough of these SNPs were confirmed. The variant allele frequencies of *ACE* rs4343A>G SNP in Thai population in this study were lower than those reported in Caucasian populations, indicating that Thai subjects with rs4343A>G polymorphism might have higher incidence of cough from ACE inhibitors usage than Caucasian subjects.

A previous study in Spanish population reported the association between a variation in *ACE* gene (rs4344A>G) and ACE inhibitor-induced cough.[18] A protective effect of rs4344A>G polymorphism against cough due to ACE inhibitors was found in male patients. However, the risk of cough related with ACE inhibitor therapy was higher in female patients.[18] Our study observed that males had 162% higher serum ACE activity than females. This might be due to the effect of estrogen in decreasing ACE activity and the effect of testosterone on ACE activity is otherwise.[28] Thus inhibition of ACE activity leading to cough was more pronounced in females than in males. Our study in Thai population also showed the impact of *ACE* polymorphism (rs4344A>G) on serum ACE activity. A significantly higher serum ACE activity was observed in participants with GG genotype compared to those with AA genotype. Therefore rs4344A>G variants might protect Thai subjects from ACE inhibitor-induced cough. G allele frequency was noticed more frequently in Caucasians and Africans than in Thais. Hence, the protective effect of cough from ACE inhibition might be detected in Caucasians and Africans more than in Thai populations.

The results also showed that the effect of *ACE* rs4362C>T polymorphism on serum ACE activity in Thai population was similar to other *ACE* SNPs in this study. Thai individuals with *ACE* rs4362T homozygosity had higher median serum ACE activity than individuals with *ACE* rs4362C homozygosity. This supported the findings from previous GWS studies in Japanese subjects.[15,19] The plasma ACE activity was significantly affected by rs4362C>T polymorphism.[19] In addition, *ACE* rs4362C>T SNP strongly correlated to ACE levels in plasma.[15] A lower variant allele frequency for *ACE* rs4362C>T SNP was observed in Thais compared with Caucasians, indicating that the incidence of cough due to ACE inhibitors might vary between these two populations.

Conclusion

In conclusion, the current study revealed the effects of *ACE* rs4311C>T, rs4343A>G, rs4344A>G and rs4362C>T polymorphisms on ACE activity in Thai population which resulted in increased serum ACE activity. In addition, there were differences in variant allele frequencies of *ACE* rs4311C>T, rs4343A>G and rs4344A>G SNPs between Thais and those previously reported in other populations. Further investigations are required to evaluate the protective effects of these polymorphisms against cough due to inhibition of ACE activity in Thai patients who use ACE inhibitors.

Acknowledgements

The authors gratefully acknowledge the financial support provided by Faculty of Medicine, Thammasat University Research Scholar, Contract No 2-20/2561. We would also like to thank all healthy Thai participants in this study and my colleagues.

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