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Original research article

Impact of *ABO* rs505922 Genetic Variant on Angiotensin-Converting Enzyme Activity in Thai Population

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ABSTRACT

ABO polymorphisms have been reported to associate with angiotensin converting enzyme (ACE) inhibitor-induced cough and ACE activity. This study aimed to investigate frequencies of *ABO* rs505922C>T SNP in Thais and compare them to that in other ethnicities. The impact of this SNP on ACE activity in the Thai population was also determined. Genomic DNA from 100 healthy Thai volunteers was isolated from whole blood and genotyping. The serum ACE activity was assessed. Significant differences in T allele frequencies for rs505922C>T were noticed between Thais (0.48) and Caucasians (0.65) ($p = 0.022$). However, the frequency of T allele was not significantly different between Thai and Japanese populations (0.55) ($p = 0.396$). Thai subjects with TT genotype had significantly lower serum ACE activity (median: 26 U/L; n=25) than subjects with CT genotype (median: 32 U/L; n=45) ($p = 0.018$). The impact of this SNP was significant in females ($p = 0.021$). Moreover, serum ACE activity tended to be lower in subjects with TT genotype compared to CC genotype (median: 29, U/L; n=30) ($p = 0.480$). The *ABO* rs505922C>T has an impact on serum ACE activity in the Thai population. There were variant allele frequency differences between Thais and Caucasians. Clinical trials for Thai patients with ACE inhibitor-induced cough are required to evaluate the effects of this SNP on ACE activity.

Keywords: ABO; ACE; Angiotensin converting enzyme activity; Polymorphism; rs505922

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1. Introduction

Angiotensin-converting enzyme (ACE) inhibitors are widely prescribed for the treatment of cardiovascular diseases. The adverse drug reactions are usually mild. However, severe adverse reactions may occur such as angioedema, renal insufficiency and persistent dry cough leading to discontinuation of therapy [1]. ACE inhibitor-related cough has occurred in 5% to 35 % of patients treated with this drug [2]. Interestingly, high incidence of cough in Thai patients after receiving imidapril and enalapril therapy has been reported at 44% and 66%, respectively [3]. Ethnic differences also influence the rates of discontinuation of ACE inhibitors due to cough. Black patients discontinue taking drugs with a higher incidence (9.6/100) than non-black patients (2.4/100) [4].

Pathophysiology of ACE inhibitor-related cough has been proposed. Bradykinin and substance P were destroyed by ACE. ACE inhibition by ACE inhibitors increased accumulation of bradykinin and substance P in the respiratory tract. Bradykinin and substance P stimulated C-fibers through type J receptors causing cough reflex [5]. ACE properties such as intracellular targeting, water solubility, and degradation by lectin were determined by glycosylation [6]. In addition, glycosylation defined ABO blood types. Glycosyltransferase enzyme is encoded by the ABO gene. The association between *ABO* polymorphisms and ACE activity has been reported [7]. Moreover, variation in the *ABO* gene was related to cough, a side effect of ACE inhibitors [8-9].

The rs505922C>T, a single nucleotide polymorphism (SNP) of the *ABO* gene is located on chromosome 9. Genome-wide association studies in the Japanese population showed a significant relationship between *ABO* rs505922C>T SNP and plasma ACE activity. Haplotypes of rs505922, rs8176746 and rs8176750 have been associated with ABO blood group antigens and plasma ACE activity in people of Northern and Western European descent [7]. There has been no study about *ABO* rs505922C>T polymorphism and ACE activity in the Thai population. Therefore, the objective of this study was to investigate the frequency of rs505922C>T SNP in the Thai population and to compare it with that previously reported in other populations. Association between the *ABO* rs505922 and serum ACE activity in the Thai population was also assessed.

2. Materials and Methods

2.1 Study population

Healthy Thai male and female subjects (aged 19-45 years) were permitted to enter this study. Subjects with any of the following conditions were excluded: hypertension, heart disease, thyroid disease, diabetes mellitus, pulmonary disease, cirrhosis, leprosy, cancer and AIDS. Other exclusion criteria were pregnancy, nursing mother, smoking, alcohol or drug abuse. Steroid medications and herbs were ruled out from 2 weeks before the study. Ethical approval was obtained from Human Research Ethics Committee of Thammasat University No.1 (Faculty of Medicine), Thailand.

2.2 Blood sampling

Venous blood samples were collected then centrifuged (3,500 rpm; 10 min.; 4°C) after standing at room temperature for 1 hour to obtain serum. Serum for ACE activity assessment was kept at -20°C. Whole blood for DNA isolation was drawn by venipuncture into EDTA-coated tubes and stored at 2-8°C.

2.3 Measurement of serum angiotensin-converting enzyme (ACE) activity

Serum ACE activity was measured by a spectrophotometer (Shimadzu, Japan). This assay was performed as described by Ronca-Testoni et al. [10]. The furanacryloyl-L-phenylalanylglycylglycine (FAPGG) (Sigma-Aldrich, USA) is used as a substrate for angiotension converting enzyme. The 1

mL of reaction volume comprised 50 µl of serum and 500 µl of substrate-buffer solution containing per liter: FAPGG 0.8 mL, NaCl 0.3 mol and borate 80 mmol, pH 8.2 (37 °C). Distilled water 450 µl was added to make a final volume of 1 mL. After incubation at 37^{o} C, changes in absorbance were measured at 10-min intervals for 20 minutes. One unit (U) of ACE activity is the amount of enzyme which converts FAPGG 1 µmol in to FA-Phe and Gly-Gly at 37^{o} C. ACE activity was calculated with this equation: ACE activity (U/L) = (ΔA/min x V_t x 1000)/(0.5 x V_s), where ΔA/min is changes in absorbance of FAPGG at 345 nm in 1 minute, V_t = final volume 1 mL, 0.5 is millimolar ΔA of hydrolysis FAPGG and V_s is sample volume 50 µl.

2.4 Genomic DNA extraction

Genomic DNA (gDNA) was extracted from whole blood by using QIAamp DNA blood mini kit according to the manufacturer's protocol (Qiagen, Germany). DNA concentration and purity were assessed by using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA). The DNA samples were normalized to a concentration of 20 ng/µl.

2.5 Genotyping

The *ABO* rs505922C>T SNP was genotyped based on real-time polymerase chain reaction-based allelic discrimination (Applied Biosystems, USA) using TaqMan genotyping master mix protocol. The purified gDNA was used as the template. The real-time PCR reaction mix volume was 10 µl per well. The real-time polymerase chain reaction (real-time PCR) thermal cycling program consisted of enzyme activation at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing and extension at 60°C for 60 seconds. The assay was performed in duplicate.

2.6 Statistical analysis

Deviation of observed and expected genotype frequencies from Hardy-Weinberg equilibrium (HWE) was assessed by Chi-squared test. Fisher's exact test was performed to determine differences in allele frequencies between healthy Thai volunteers and previously reported other populations. Shapiro-Wilk and Kolmogorov-Smirnov tests were used to test non-normality for data sizes < 50 and ≥ 50 respectively. Continuous data were presented as median and inter-quartile range (IQR). All categorical data were compared by using Kruskal–Wallis test and Mann–Whitney U test. A p value less than 0.05 was taken as statistically significant.

3. Results and Discussion

3.1 Results

One hundred healthy volunteers were included in this study, seventy-two participants were female and twenty-eight were male. Among the 100 subjects, genotype frequencies of *ABO* SNP rs505922C>T were 30% for homozygous wild-type, 45% for heterozygous and 25% for homozygous variant (Table 1). The observed genotype frequencies were in Hardy-Weinberg Equilibrium and did not differ from expected genotype frequencies (p = 0.328).

Table 1. Genotype frequencies of ABO rs505922C>T in healthy Thai volunteers (N=100).

Genotype	Genotype frequency (%)		χ^2	p value
	Observed	Expected		
CC	30	28	0.955	0.328
CT	45	50		
TT	25	22		

Statistical evaluation for Hardy-Weinberg Equilibrium was performed by using Chi-squared test.

The comparison of allele frequencies of *ABO* rs505922C>T among various populations is illustrated (Table 2). The C

allele frequency in Thai (0.52) was significantly higher than in European populations reported by Germain and colleagues (0.35) [11] (p = 0.022). However, there were no significant differences in allele frequencies of a wild type allele between Thais and previously reported Japanese [12] or European populations in another study [13]. The variant allele frequency for *ABO* rs505922C>T was significantly lower in Thais than in Europeans (0.48 versus 0.65) [11] (p = 0.022). However, the T allele frequency in Thais did not significantly differ from Japanese and other European populations [13].

Table 2. Comparison of allele frequencies of *ABO* rs505922C>T between healthy Thai volunteers and previously reported other populations.

Population	N	Allele frequency		p value	Ref.
		C	T		
Asian					
Thai	100	0.52	0.48		Current study
Japanese	1,639	0.45	0.55	0.396	[12]
Caucasian					
European	623	0.40	0.60	0.118	[13]
European	1,110	0.35	0.65	0.022*	[11]

Statistical evaluation was performed by using Fisher's exact test, *p < 0.05

The effects of rs505922C>T SNP of *ABO* gene on serum ACE activity is revealed (Table 3 and Fig. 1). Significantly lower serum ACE activity (median: 26, IQR: 18-36 U/L) was observed in subjects with TT genotype compared to CT genotype (median: 32, IQR: 28-40 U/L) (p=0.018). In addition, subjects with TT genotype tended to have lower serum ACE activity compared to subjects with CC genotype (median: 29, IQR: 22-42 U/L) (p = 0.480).

Table 3. Influence of *ABO* rs505922C>T SNP on serum angiotensin-converting enzyme (ACE) activity in Thai healthy volunteers (N=100).

Genotype (N)	Serum angiotensin-converting enzyme activity (U/L)		
	Median (IQR)	Minimum	Maximum
CC (30)	29 (22-42)	14	70
CT (45)	32 (28-40)	14	66
TT (25)	26 (18-36)	16	52

IQR: inter-quartile range

Female gender significantly influenced *ABO* rs505922C>T SNP and serum ACE activity (Table 4) (p = 0.021). The lowest serum ACE activity was observed in *ABO* rs505922T homozygous subjects (median: 22, IQR: 18-34 U/L; N=18) followed by *ABO* rs505922C homozygous subjects (median: 26, IQR: 22-36 U/L; N=22) and *ABO* rs505922 heterozygous subjects (median: 31, IQR: 26-40 U/L; N=32), respectively.

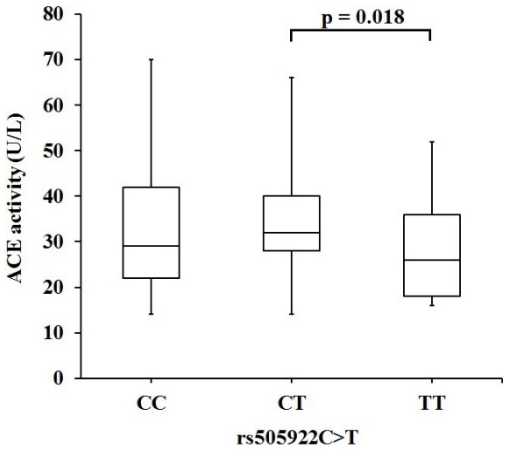

Fig. 1. Influence of ABO rs505922C>T polymorphism on serum ACE activity. Data are expressed as median and interquartile range. Statistical evaluation was performed by using by Mann–Whitney U test.

Table 4. Influence of gender on genotype frequencies and serum angiotensin converting enzyme (ACE) activity of *ABO* rs505922C>T in healthy Thai volunteers (N=100).

Gender	Genotype (%)	Serum angiotensin-converting enzyme activity (U/L)		p value
		Median	IQR	
Male	CC (8)	34	26-43	0.806
	CT (13)	40	32-44	
	TT (7)	36	29-42	
Female	CC (22)	26	22-36	0.021
	CT (32)	31	26-40	
	TT (18)	22	18-34	

Statistical evaluation was performed by using Kruskal–Wallis test, IQR: inter-quartile range

3.2 Discussion

The ABO gene is located on chromosome 9 (9q34.1-q34.2) [14], which encodes glycosyltransferase to determine ABO blood groups [15]. Besides ABO blood types, glycosylation is important for ACE synthesis [16], hydrosolubility, intracellular targeting and destruction [6]. The ABO blood group SNP rs505922C>T is located within intron 1 of the *ABO* gene [17], thus this polymorphism may influence glycosylation of ACE and consequently ACE activity. Our study reveals the impact of *ABO* genetic polymorphisms (rs505922C>T) on serum ACE activity in the Thai population. Median serum activity of ACE was significantly lower in healthy Thai subjects carrying 2 variant alleles for *ABO* rs505922C>T than in subjects carrying only one T allele. ACE inhibitor-induced cough might occur from reduced bradykinin degradation by ACE, consequently increased bradykinin level [18]. Because a decrease in ACE activity caused an increase in bradykinin concentration from ACE inhibition, [8], subjects who have lower ACE activity might have a higher risk of cough due to ACE inhibition than other genotypes.

Our study shows the impact of *ABO* rs505922 SNP on serum ACE activity which supports the previous study by Terao et al. [7]. Terao and colleagues revealed variation in rs505922 SNP influenced plasma ACE activity [7]. They selected 3 *ABO* SNPs from genome-wide association studies (GWAS) in British families of Northern and Western European descent. Haplotypes of rs505922C>T/rs8176746G>T/rs8176750G>- were constructed and revealed marked association with ABO blood groups and ACE activity in plasma. Subjects with TGG haplotype which tagged type O alleles had moderate activity of ACE and had lower plasma ACE activity compared to CTG haplotype which tagged type B alleles. In contrast, the subjects with TGG haplotype had higher plasma ACE activity than subjects with CGG haplotype which tagged type A1 alleles. The T allele of rs505922 in the haplotype analysis did not show the lowest plasma ACE activity, because this haplotype analysis was composed of 3 SNPs namely, rs505922, rs8176746 and rs8176750. All SNPs must be carried together to influence ACE activity whereas our study observed the impact of one SNP (rs505922) on ACE activity. Besides ACE activity, there have been studies to observe the relationship between rs505922 and ACE level [12]. Yamagata University Genomic Cohort Consortium (YUGCC) performed GWAS to identify genetic diversity of the ABO locus in the Japanese population. Two groups of the population were composed of 1,639 people from Takahata town located in Yamagata Prefecture and 1,672 people from prefectural capital of Yamagata city. However, lack of association of rs505922 SNP with plasma ACE levels ($p>10^{-7}$) in both Takahata and Yamagata populations was shown.

Ethnic differences may affect incidence of ACE inhibitor-induced cough [2-4]. The variant allele for *ABO* (rs505922C>T) was significantly less observed in Thais than in Caucasians as

reported by Germani et al [11]. However, there was no significant differences in variant allele frequencies for rs505922 between Thai and Japanese populations. Thus, Thai patients with TT genotype for rs505922 who take ACE inhibitors might experience less cough than Caucasians. However, experience of cough associated with using ACE inhibitors in rs505922T homozygous Thai population might not differ significantly from Japanese population with TT genotype of rs505922C>T. Moreover, gender differences also influenced *ABO* polymorphisms and ACE activity. Our study shows the influence of *ABO* rs505922 SNP on serum ACE activity only in females. The explanation might be that estrogen decreases ACE activity whereas testosterone increases ACE activity. Women after puberty had lower ACE activity than male adults [19]. The incidence of ACE inhibitor-induce cough in Spanish patients was significantly increased in females ($p = 0.0001$) [9]. Thus females with *ABO* rs505922T homozygosity were suspected to face increased risk of ACE inhibitor-related cough.

4. Conclusion

The ABO blood group SNP rs505922C>T assessed in this study influenced serum ACE activity in the Thai population. There were differences in the frequency of functional variants between Thais and Caucasians. Further studies are required to confirm the influence of this polymorphism on serum ACE activity in Thai patients treated with ACE inhibitors.

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