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Original Article

Angiotensin-converting enzyme (ACE) insertion/deletion polymorphism is associated with dry eye syndrome (DES) in Korean population

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Abstract: Objective: The aim of this study was to determine if angiotensin-converting enzyme (ACE) gene was associated with dry eye syndrome (DES) in Korean population. Materials and methods: 153 DES patients and 45 control subjects were enrolled in this study and DES patients were divided into meibomian gland dysfunction (MGD) and aqueous tear-deficient dry eye (ADDE) groups according to etiology. ACE insertion/deletion polymorphism was analyzed using polymerase chain reaction (PCR) and 1.8% agarose gel electrophoresis. The genotypes were classified into three according to the length of the PCR product: I/I (490 bp band), D/D (190 bp band), and I/D (490 bp band and 190 bp band). SNPStats and SPSS 18.0 were used for genotype and allele analysis. Logistic regression models were conducted for odds ratio (OR), 95% confidence interval (CI), and *P* value. Results: Genotypic frequencies of I/I, I/D, and D/D revealed 34.6%, 53.6%, and 11.8% vs. 24.4%, 53.3%, and 22.2% (DES group vs. control group), respectively. In analysis of DESs, ACE insertion/deletion polymorphism revealed significant association with MGD [OR = 0.24, 95% CI = 0.07-0.87, P = 0.025 in the recessive model (I/I+I/D vs. D/D) and ADDE [OR = 0.23, 95% CI = 0.07-0.78, P = 0.018 in the codominant 2 model (I/I vs. D/D); OR = 0.30, 95% CI = 0.10-0.88, P = 0.026 in the recessive model (I/I+I/D vs. D/D); OR = 0.51, 95% CI = 0.28-0.92, P = 0.022 in log-additive model (I/I vs. I/D vs. D/D)]. Conclusions: These data suggest that ACE insertion/deletion polymorphism may be associated with susceptibility to MGD and ADDE in DESs in the Korean population.

Keywords: Association study, dry eye syndrome, ace, insertion/deletion polymorphism

Introduction

Dry eye syndrome (DES) is defined as tear film disorder due to tear deficiency or excessive evaporation [1]. It represents symptoms of discomfort, visual disturbances, tear film instability, accompanied by increased tear film osmolarity and inflammation of ocular surface [2]. DES is a common disease. It is estimated that a significant number of elderly people in the United States have symptoms of DES [3]. This is more prevalent in women, and DES, that causes clinical symptoms and severe symptoms, is commonly found in American women over middle age [4]. This is not so different in Korea. Overall prevalence of diagnosed DES in Korea in 2010-2011 was 8.0%, and prevalence of DES symptoms was 14.4% [5].

DES can be categorized largely according to its etiology, such as aqueous tear-deficient dry eye (ADDE) lacking tears and evaporative dry eye (EDE) with excessive tear evaporation. ADDE includes Sjögren syndrome (SS) and non-Sjögren syndrome (nSS) dry eye, and EDE includes dry eye due to meibomian oil deficiency, drug abuse, malnutrition, and so on [6]. DES by autoimmune disease SS is due to characteristic inflammatory change, with presence of lacrimal gland and conjunctival inflammatory mediators [7]. DES by nSS revealed conjunctival inflammation caused by inflammatory cell infiltration and upregulation of expression in immunostimulatory markers [8]. MGD is one of the most common causes of EDE and has a relationship with inflammation, microbial factors and lipid deficiencies [9].

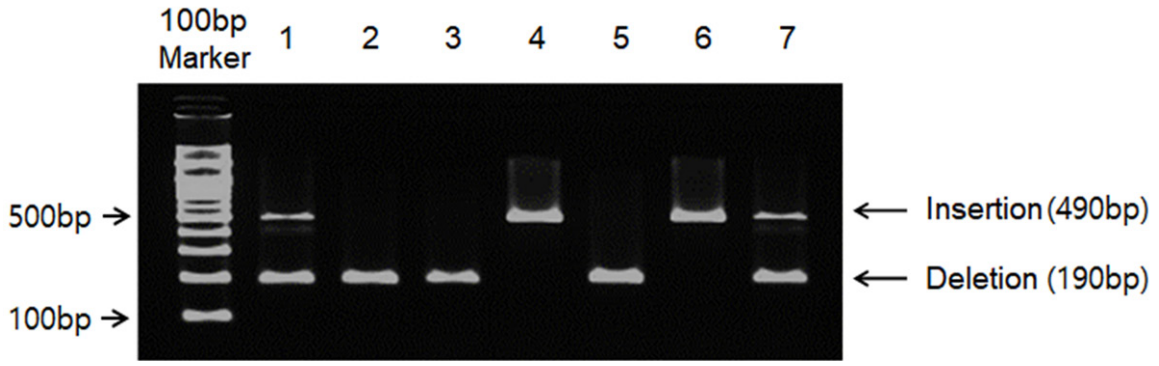

Figure 1. Determination of *ACE* insertion/deletion polymorphism on 1.8% agarose gel. Lane: 1,7-heterozygous insertion/deletion genotype; 2,3,5-homozygous deletion/deletion genotype; 4,6-homozygous insertion/insertion genotype.

Angiotensin-converting enzyme (ACE) is a gene closely related to inflammation. ACE functions to convert angiotensin I to angiotensin II (Ang II). Ang II is one of the most crucial components of the renin-angiotensin system and has proinflammatory action. Ang II stimulates expression of nuclear factor-kappa B, a transcription factor that regulates gene expression in inflammatory cytokines [10]. Therefore, there are many studies of ACE function and inflammatory diseases. Inhibition of ACE enhances neutrophil, eosinophil, basophil and mononuclear cell infiltration in asthma [11]. In gastric mucosa, ACE was regulated by H. pylori and was associated with gastrointestinal inflammation [12]. ACE was associated with high-sensitivity C-reactive protein, metalloprotease 9, interleukin-6 and platelet aggregation in coronary artery disease [13]. Insertion (I)/deletion (D) polymorphism in the *ACE* gene refers to the Alu repeat sequence in intron 16. This polymorphism is associated with levels of ACE in plasma and tissues [14]. Therefore, the relationship between I/D polymorphism and various inflammatory diseases has been studied extensively, and diseases such as periodontal disease [15], atherosclerosis [16], asthma [17], vitiligo [18] etc.

The purpose of this experiment is to examine the relationship between *ACE* insertion/deletion polymorphism and susceptibility of DES in Korean population.

Materials and methods

Subjects

Dry eye patients were recruited from our dry eye clinic at the Department of Ophthalmology, Dankook University at Cheonan. The sample of dry eye disease included established patients of dry eye disease that visited our clinic between August 2016 and June 2017. The diagnostic was based on clinician's standard clinical practices: (1) a reporting of symptoms: burning sensation, irritation, grittiness or foreign body sensation, light sensitivity, pain, dryness, soreness, or discomfort in the eye; (2) a non-anesthesia Schirmer I value of < 10 mm/5 minutes. or (3) positive corneal staining. or (4) fluorescein tear break up time of < 10 seconds (The inclusion criteria of DES). Diagnostic criteria of MGD were as follows: (1) symptoms of ocular discomfort: (2) clinical signs: at least 1 lid margin feature (plugging, capping, vascularity), abnormal meibum quality (The inclusion criteria of MGD). Control patients that visited our clinic with refraction-related complaints or cataract or for eye examination were recruited for this study. Potential subjects were excluded from the study if they had undergone previous corneal or ocular surgery, had ocular pathology other than dry eye disease, or had systemic diseases affecting the eye (The exclusion criteria). According to the above criteria, participants were divided into DES group (all DES patients), MGD group (patients with MGD among DES patients), ADDE group (patients with ADDE among DES patients), and control group (no DES, patient with other eye disease). All participants signed an informed consent form in accordance with tenets of the Declaration of Helsinki and this study was approved by the Institutional Review Board of Dankook University Hospital, Cheonan, Korea.

DNA extraction and genotyping

We extracted genomic DNA from oral buccal cells of subjects [15]. Before physically collect-

Table 1. Demographic information of subjects

	Controls	DES	χ^2	T	*P* value
Total	45	153			
Male/female	26/19	60/93			
Age (mean ± SD)	38.8 ± 15.2	55.1 ± 14.0			
DM (absence/present)	43/2	137/16	1.129		0.288
HTN (absence/present)	39/6	111/42	4.013		0.045
Sjögren's syndrome (absence/present)	45/0	146/7	2.178		0.140
MGD (absence/present)	45/0	82/71	33.496		0.000
Marx's line (absence/present)	45/0	101/52	21.273		0.000
Shimer test (mean ± SD)	9.96 ± 3.93	7.63 ± 5.57		1.245	0.215
BUT (mean ± SD)	6.89 ± 1.85	4.23 ± 1.57		4.849	0.000
OSDI (mean ± SD)	17.30 ± 17.34	37.09 ± 21.20		-5.688	0.000
Corena staning (mean ± SD)	0.67 ± 0.97	2.27 ± 2.62		-1.812	0.072
Conjunctival staining (mean ± SD)	0.78 ± 1.03	2.03 ± 1.69		-2.185	0.030

Inappropriate clinical data was excluded for the exact analysis. DES, dry eye syndrome; DM, diabetic mellitus; HTN, hypertension; MGD, meibomian gland dysfunction; BUT, tear film breakup time; OSDI, rasch analysis of the ocular surface disease Index.

ing cells inside of a patient's mouth, we confirmed that there was no substance that could result in complications with DNA typing methods by requiring the subject to wash the mouth with water. We collected epithelial cells from the buccal region using sterilized swabs. Genomic DNA was extracted according to the protocol of the DNA extraction kit (Roche, Mannheim, Germany). Genotype for *ACE* insertion/deletion polymorphism was determined by polymerase chain reaction (PCR) [15, 19], using sense primer 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and anti-sense primer 5'-GAT GTG GCC ATC ACA TTC GTC AGA-3'. PCR was conducted using commercial PCR mastermix kit (NanoHelix, Daejeon, Korea). PCR conditions were as follow: 1) initial denaturation at 94°C for 5 minutes 2) 39 cycles at 94°C for 30 seconds, 58°C for 40 seconds, and 72°C for 1 minute 3) final elongation step of 72°C for 10 minutes. PCR products were analyzed by 1.8% agarose gel electrophoresis and ethidium bromide staining to identify three genotypes: I/I (490 bp band), D/D (190 bp band), and I/D (490 bp band and 190 bp band) (**Figure 1**).

Statistical analysis

The prevlences of DM, HTN, Sjögren's syndrome, MGD, and Marx's line (absence/present) in each group were analyzed by Chi square test, and clinical observations related to DES were analyzed by independent t-test. SPSS 18.0 was used for the analysis (SPSS Inc., Chicago, IL, USA). Multivariate logistic regression was performed using codominant 1 (I/I *vs*. I/D), codominant 2 (I/I *vs*. D/D), dominant (I/I *vs*. I/D+D/D), recessive (I/I+I/D *vs*. D/D), and log-additive models (I/I *vs*. I/D *vs*. D/D) to determine differences in ACE I/D genotype frequencies between the DES group and the control group [20-22]. SNPStats and SPSS 18.0 were used for statistical analysis. Logistic regression was conducted to evaluate odds ratio (OR), 95% confidence intervals (CI), and *P* value. For statistical tests, the level of significant *P* value was set at 0.05.

Results

Clinical symptoms including presence of MGD, Marx's line and level of BUT, OSDI, and conjunctival staining are related to DES

There were no significant differences between the groups in DM, HTN, Sjögren's syndrome, MGD, and Marx's line. However, there was slightly statistically significant in HTN. The presence of MGD, Marx's line and level of BUT, OSDI, and conjunctival staining were statistically significant. There was no statistical difference in Shimer test and corena staining results between DES group and control (**Table 1**).

Genotype distributions

In this study, we investigated if insertion/deletion polymorphism in the *ACE* gene was associated with susceptibility to DES. The 153

Table 2. Genotype frequencies of the ACE insertion/deletion polymorphism in controls and patients with dry eye syndrome (DES)

Genotype/allele	Control n (%)	DES n (%)	Models	OR (95% CI)	*P* value
I/I	11 (24.4%)	53 (34.6%)	Codominant 1	0.79 (0.32-1.97)	0.40
I/D	24 (53.3%)	82 (53.6%)	Codominant 2	0.33 (0.10-1.05)	0.06
D/D	10 (22.2%)	18 (11.8%)	Dominant	0.64 (0.27-1.53)	0.31
			Recessive	0.39 (0.14-1.03)	0.06
			Log-additive	0.59 (0.33-1.06)	0.08
I	46 (51.1)	188 (61.4)		1	
D	44 (48.9)	118 (38.6)		0.66 (0.41-1.05)	0.08

Logistic regression analysis was performed. ACE, angiotensin-converting enzyme; OR indicates odds ratio; CI, confidence interval; DES, dry eye syndrome.

Table 3. Genotype frequencies of the *ACE* insertion/deletion polymorphism in patients with MGD

Genotype/allele	ControIn (%)	MGDn (%)	Models	OR (95% CI)	*P* value
I/I	11 (24.4%)	22 (31.0%)	Codominant 1	0.86 (0.27-2.55)	0.77
I/D	24 (53.3%)	42 (59.1%)	Codominant 2	0.22 (0.05-0.97)	0.09
D/D	10 (22.2%)	7 (9.9%)	Dominant	0.66 (0.23-1.84)	0.42
			Recessive	0.24 (0.07-0.87)	0.025
			Log-additive	0.51 (0.24-1.06)	0.06
I	46 (51.1)	86 (60.6)		1	
D	44 (48.9)	56 (39.4)		0.68 (0.40-1.16)	0.16

Logistic regression analysis was performed. ACE, angiotensin-converting enzyme; OR indicates odds ratio; CI, confidence interval; MGD, meibomian gland dysfunction.

patients with DES and 45 control subjects were tested. **Table 2** revealed genotypic frequencies of *ACE* insertion/deletion polymorphism in the DES group and the control group. The genotypic frequencies of I/I, I/D, and D/D in *ACE* gene revealed 34.6%, 53.6%, and 11.8% *vs*. 24.4%, 53.3%, and 22.2% (DES group *vs*. control group), respectively. In the control group, genotype distributions of *ACE* insertion/deletion polymorphism were in Hardy-Weinberg equilibrium (P = 0.77, data not shown) (**Table 2**).

The susceptibility of MGD and ADDE is associated with ACE I/D polymorphism

In genotypic model analysis (codominant 1, codominant 2, dominant, recessive, and log-additive models), the difference of genotypic frequency between the DES group and the control group did not show significant association with susceptibility of DES (**Table 2**). We conducted additional analyses according to clinical symptoms in patients with DES (SS, MGD, and ADDE). We observed ACE polymorphism exhibited significant association with MGD and ADDE. MGD was associated with *ACE* insertion/deletion polymorphism in recessive model (I/I+I/D *vs*. D/D) (OR = 0.24, 95% CI = 0.07-0.87, P = 0.025) (**Table 3**). ADDE was associated with *ACE* insertion/deletion polymorphism in codominant 1 model (I/I *vs*. D/D) (OR = 0.23, 95% CI = 0.07-0.78, P = 0.018), recessive model (I/I+I/D *vs*. D/D) (OR = 0.30, 95% CI = 0.10-0.88, P = 0.026), and log-additive model (I/I vs. I/D vs. D/D) (OR = 0.51, 95% CI = 0.28-0.92, P = 0.022), respectively (**Table 4**). However, we did not find any relationship with SS in DESs (P > 0.05, data not shown).

Discussion

DES is triggered by a variety of causes in a wide range of people. ADDE can be caused by SS and nSS and MGD by eyelid inflammation, conjunctival inflammation, corneal injury, microbiological changes, and tear film instability [23]. Among these causes, inflammation accounts for a large proportion [24]. ADDE by SS

Table 4. Genotype frequencies of the *ACE* insertion/deletion polymorphism in patients with ADDE

Genotype/allele	Control n (%)	ADDE n (%)	Models	OR (95% CI)	*P* value
I/I	11 (24.4%)	29 (37.7%)	Codominant 1	0.66 (0.28-1.56)	0.35
I/D	24 (53.3%)	42 (54.5%)	Codominant 2	0.23 (0.07-0.78)	0.018
D/D	10 (22.2%)	6 (7.8%)	Dominant	0.54 (0.24-1.22)	0.13
			Recessive	0.30 (0.10-0.88)	0.026
			Log-additive	0.51 (0.28-0.92)	0.022
I	46 (51.1)	100 (64.9)		1	
D	44 (48.9)	54 (35.1)		0.57 (0.33-0.96)	0.034

Logistic regression analysis was performed. ACE, angiotensin-converting enzyme; OR indicates odds ratio; CI, confidence interval; ADDE, aqueous tear-deficient dry eye.

is an autoimmune disease in which the lacrimal gland becomes a major target organ and lymphocyte infiltration occurs [25]. In ADDE by nSS, inflammatory cells such as Langerhans cell and leukocyte in corneal epithelial layer were observed to change [26]. MGD is a terminal duct obstruction with various causes, one of which is inflammation. Blepharitis, and meibomitis are included in MGD-related diseases [27]. MGD is associated with SS, an increase in tear evaporation is often associated with changes in the ocular surface in SS patients [28].

Current treatment of DES includes symptomatic treatment as well as anti-inflammatory drug such as corticosteroids, immunosuppressants, etc. Diquafosol improves symptoms of ADDE in SS and nSS patients [29]. Matrix metalloproteinase 9 and transglutaminase 2 increased in ADDE by SS and MGD, decreased by corticosteroids and clinical symptoms improved [30]. Topical cyclosporine, immunosuppressants, has been used in treatment of MGD as well as ADDE [31, 32].

RAS was initially known to be involved only in blood pressure, but it also plays a significant role as proinflammatory mediator [33] and is present in the lacrimal gland [34]. RAS and its component, Ang II, play critical roles in the process and persistence of various inflammatory diseases. Since angiotensin converting enzyme is crucial for formation of Ang II encoded by the *ACE* gene, there have been many studies on variation of the *ACE* gene. Deletion polymorphism of the *ACE* gene increased serum ACE activity and risk for coronary artery disease in Japanese subjects [35]. ID/DD genotype and the D allele of the *ACE* gene were associated with risk of type 2 diabetes mellitus and hypertension in a Saudi Arabian population [36]. The presence of *ACE* D allele increased the risk of hypertension in end stage renal disease patients [37]. DD genotype of *ACE* gene was a risk factor for coronary artery disease in a north Indian population [38]. In periodontal disease, D allele increased risk of disease [15]. Since the genotype of *ACE* gene determines the level of ACE in the blood [14], many previous studies had been conducted in relation to occurrence of the disease.

In this study, we examined the association between risk of DES and ACE I/D polymorphism. To further clarify the relationship, DES patients with local factors such as systemic disease or trauma etc. were removed and statistically significant results were obtained in ADDE and MGD group (**Tables 3** and **4**). As described above, D allele of *ACE* polymorphism is a risk factor in most inflammatory diseases. Interestingly, to the contrary, our results suggest that I allele of ACE polymorphism is a risk factor in ADDE and MGD. Of course, other diseases in which I allele is a risk factor have been reported. In ruptured intracranial aneurysms, I allele increased risk of disease [29]. II genotype was associated with increased risk of Alzheimer's dementia [39].

It is not certain if the polymorphism of ACE increases susceptibility to DES, or if it causes DES to easily aggravate after occurrence. However, in this study, we revealed that ACE I/D polymorphism may have a relationship with development of MGD and ADDE. ACE I/D polymorphism could be further studied as a target for DES therapy or prevention in the future.

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Disclosure of conflict of interest

None.

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