ACE Insertion/Deletion (I/D) Polymorphism and Macroalbuminuria among Thai Patients with Type 2 Diabetes, A Single Tertiary Center Study

Patinut Buranasupkajorn MD*, Natnicha Houngngam MD**,***,
Pisut Katavetin MD****, Thiti Snabboon MD**,***

** Division of Hospital and Ambulatory Medicine, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
*** Hormonal and Metabolic Disorders Research Unit, Excellence Center for Diabetes, Hormone, and Metabolism, Faculty of Medicine, King Chulalongkorn Memorial Hospital, Bangkok, Thailand*
**** Division of Endocrine and Metabolism, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
***** Division of Nephrology, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

Objective: *To investigate the association between ACE gene (I/D) polymorphism and the presence of macroalbuminuria among Thai patients with type 2 diabetes.*
Material and Method: *A cross-sectional case control study included unrelated 188 Thai subjects with type 2 diabetes. Study subjects having diabetes for at least 10 years with normoalbuminuria and serum creatinine not exceeding 1.2 mg/dL were assigned as a control group (n = 94), and the study group was composed of type 2 diabetic patients who had macroalbuminuria (n = 94). The presence of ACE gene polymorphism was determined by PCR-gel electrophoresis.*
Results: *The distribution of II, ID and DD polymorphism did not significantly differ between study and control groups (II: 20.2%, ID: 40.4%, DD: 39.4%; and II: 25.5%, ID: 43.6%, DD: 30.9%, respectively). There was also no significant difference between allele frequencies of the I allele: 40.4% and 47.3% and the D allele in the study and control group: 59.6% and 52.7%, respectively.*
Conclusion: *A higher but not statistically significant frequency of D allele did not support an association in the I/D polymorphism of the ACE gene and macroalbuminuria in patients with type 2 diabetes. Further studies are required to identify genetic risk factors of DN in Thai patients.*

Keywords: *Macroalbuminuria, ACE gene, I/D polymorphism, Diabetic nephropathy*

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Diabetic nephropathy (DN) is the major determinant of morbidity and mortality among diabetic patients. It also relates to heavy health-economic burden. From the Thailand Diabetes Registry (TDR) project, it showed the prevalence of DN among type 2 diabetes in Thai patients at 42.9%[1]. Its clinical manifestation is characterized by a progressive increase in albuminuria, declining renal function concurring with elevated blood pressure and eventually end-stage renal disease (ESRD)[2]. It is widely accepted that DN is a heterogeneous disorder caused by an interaction between environmental and genetic factors. Modifiable risk factors including hypertension, smoking, and poorly controlled and long-term duration of diabetes are reported to be associated with DN development. Genetic factors have also been considered as an important determinant of the susceptibility and severity of DN, supporting by familial aggregation and ethnic-specific prevalence rates[3].

Various genes have been regarded in contribution to DN; however, no major susceptibility genes have been identified, so far[4]. The renin-angiotensin aldosterone system (RAAS) is believed to play an important role in the pathogenesis of DN: regulating blood pressure, electrolyte imbalance and renal hemodynamics. Angiotensin-converting enzyme

Correspondence to:
Buranasupkajorn P, Division of Hospital and Ambulatory Medicine, Department of Medicine, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Rama IV Road, Pathumwan, Bangkok 10330, Thailand.
Phone: +66-91-4494373
E-mail: patinutbur@yahoo.com.sg

(ACE) gene, the key role in catalyzing the conversion of angiotensin I to angiotensin II, is proposed as the one of plausible factors. It locates on the long arm of chromosome 17 (17q23) containing 26 exons, which the insertion (I)/deletion (D) polymorphism of a 287-bp Alu1 element (rs1799752) inside intron 16 produces three genotypes: II, ID, and DD[5]. From several epidemiological studies, an association between this polymorphism and the development and progression of DN has been shown, especially in Asian population[6]; however, the difference between ethnicities was noted. The purpose of the present case-control study was to investigate the association of this ACE I/D polymorphism and the presence of macroalbuminuria among our diabetic patients, which could be used as a predictor for the development and progression of their nephropathy.

Material and Method

This case-control study was recruited from all unrelated diabetic participants, 94 case patients and 94 control subjects, attended the outpatient clinic of King Chulalongkorn Memorial Hospital. They were all diagnosed as type 2 diabetes according to 1998 World Health Organization diagnostic and classification criteria. Subjects who had duration of diabetes of at least 10 years with normoalbuminuria and serum creatinine not exceeding 1.2 mg/dL were assigned as control subjects. The study group was composed of type 2 diabetes patients who had a spot albumin to creatinine excretion ratio (ACR) of at least 300 mg/day in two of three consecutive specimens. Albuminuria measurements were performed with immunoturbidimetric assay on Cobas 6000 Analyzer (Roche Diagnostics). All subjects with other renal diseases or having proteinuria from other causes such as microscopic hematuria, urinary stone or tumor, HIV or hepatitis B or C infection were excluded. The presence of microalbuminuria was not included in the present study due to its reversible condition and ease of disturbance from several factors. Anthropometric data and details of biochemical parameters including fasting plasma glucose, HbA1c, serum creatinine, lipid profile and urine analyses from each subject were collected and analyzed. Written informed consents were obtained from all participants. The present study was approved by the Ethics Committee, Faculty of Medicine, Chulalongkorn University.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by standard procedures with a DNA Mini Kit (QIAGEN GmbH, Kilden, Germany). Genotyping for ACE I/D polymorphism was using a Polymerase Chain Reaction (PCR) method with appropriate primers and conditions previously described[5]. Each sample having the DD genotype was re-amplified with an insertion-specific sequence[7]. The products were separated by electrophoresis on 1% agarose gels to detect a 190 bp fragment in the absence of insertion (D), and a 490 bp fragment in the presence of insertion (I) in the ACE gene.

Statistical analysis

The student's t-test or Wilcoxon rank-sum test was used to compare quantitative baseline data, and Pearson's Chi-square test or Fisher's exact test was used for category baseline data. Primary analysis was performed by Pearson's Chi-square test to compare genotype and allele distribution between groups. Sensitivity analyses included unadjusted and adjusted logistic regression analyses of three genetic models (additive, dominant, and recessive models). The adjusted model included age, sex, duration of diagnosed diabetes, hypertension, systolic blood pressure, use of ACE inhibitors or angiotensin receptor blockers (ARB), HbA1c level and smoking status. Statistically, a *p*-value of less than 0.05 was considered statistically significant. All statistical analyses in the study were performed with STATA software, version 14.

Results

Demographic and clinical features of the subjects from both groups were shown in Table 1. The median duration of DN among the study group was 2 years, range from 1 to 5 years. There were significantly higher in male patients, BMI, diagnosis of hypertension, systolic blood pressure, HbA1C, serum creatinine, albumin/creatinine ratio, triglyceride, ACEI or ARB use, any diabetic retinopathy and diagnosis of ischemic heart disease in the study group compared with the control group ($p<0.05$). However, higher HDL-C level was found in the control group.

Genotype distribution of our study population was in agreement with Hardy-Weinberg Equilibrium (HWE). Table 2 showed the ACE I/D genotype distribution in control and study groups. Among the total patients, 43 (22.9%) patients had the II genotype, while the ID genotype was present in 79 (42.0%) patients and 66 (35.1%) patients with the DD genotype. In the study group, the frequencies of the II,

Table 1. Clinical and biochemical data in cases and controls

	Control (n = 94)	Macroalbuminuria (n = 94)	*p*-value
Age, years	63.9±8.7	65.4±9.8	0.27
Male, n (%)	28 (29.8%)	44 (46.8%)	0.02
Duration of diabetes, years*	15.5 (12, 19)	14 (9, 20)	0.06
BMI, kg/m^2	25.7±5.2	28.1±4.8	<0.01
Hypertension, n (%)	78 (83.0)	88 (93.6)	0.02
Systolic blood pressure (mmHg)	131.4±14.8	138.2±18.4	<0.01
Diastolic blood pressure (mmHg)	72.9±8.3	74.0±11.6	0.46
Fasting plasma glucose (mg/dL)	132.1±40.9	139.5±44.4	0.24
HbA1C (%)	7.34±1.16	7.86±1.30	<0.01
Serum creatinine (mg/dL)	0.80±0.20	1.30±1.05	<0.01
Albumin/creatinine ratio (mg/g)*	7.5 (4.7, 13.0)	732.6 (513, 1,061.5)	<0.01
Total cholesterol (mg/dL)	164.6±31.7	169.1±35.9	0.37
Triglyceride (mg/dL)	116.0±65.1	142.8±67.8	<0.01
HDL-c (mg/dL)	53.8±13.0	49.6±15.7	0.04
LDL-c (mg/dL)	87.6±27.9	90.9±32.1	0.45
Ever smoking, n (%)	21 (22.3%)	30 (32.3%)	0.13
Family history of kidney disease, n (%)	9 (9.6%)	3 (3.3%)	0.08
ACEI or ARB use, n (%)	74 (78.7%)	86 (91.5%)	0.01
Any diabetic retinopathy, n (%)	27 (29.0%)	44 (47.8%)	<0.01
Ischemic heart disease, n (%)	4 (4.3%)	14 (14.9%)	0.01
Stroke, n (%)	5 (5.3%)	9 (9.6%)	0.27

Mean ± SD results for normal distributed variable and * Median, interquartile range and Wilcoxon rank-sum test results for non-normal distributed variable.
N = Number of individuals; DN = diabetic nephropathy; BMI = body mass index; HDL-c = high-density lipoprotein cholesterol; LDL-c = low-density lipoprotein cholesterol; ACEIs = Angiotensin I converting enzyme inhibitor; ARB = angiotensin receptor blocker

Table 2. Genotype and allele frequencies of the ACE I/D polymorphism in the control and study group

ACE gene	Controls (n = 94)	Macroalbuminuria (n = 94)	*p*-value
Genotype			0.435
DD	29 (30.9%)	37 (39.4%)	
DI	41 (43.6%)	38 (40.4%)	
II	24 (25.5%)	19 (20.2%)	
Allele			0.177
Deletion	99 (52.7%)	112 (59.6%)	
Insertion	89 (47.3%)	76 (40.4%)	

ID and DD genotypes were not statistically significant at 20.2%, 40.4%, and 39.4%, respectively, compared to 25.5%, 43.6%, and 30.9% in the control group, respectively. The prevalence of total D allele was slightly higher in the study group (59.6%) compared with the control group (52.7%), but without statistical significance. With the logistic regression analyses of additive, dominant and recessive model of macroalbuminuria with the D allele or DD genotype were not significantly higher than those without the allele or genotype as shown in Table 3.

Discussion

This cross-sectional case-control study demonstrated a higher but not significantly different frequency of the D allele of the ACE I/D polymorphism

Table 3. Logistic regression analyses of three genetic models

	OR and 95% CI for macroalbuminuria		
Genetic models	Additive model	Dominant model	Recessive model
Unadjusted model	1.28 (0.87, 1.88)	1.35 (0.68, 2.68)	1.45 (0.80, 2.66)
Adjusted model*	1.25 (0.82, 1.91)	1.37 (0.65, 2.89)	1.38 (0.71, 2.67)

* Adjusted for age, sex, duration of diagnosed diabetes, A1c level, hypertension, systolic blood pressure, use of ACE inhibitors or ARB and smoking status

in the presence of macroalbuminuria among our T2DM patients. We also could not demonstrate any association between the ACE I/D polymorphism and macroalbuminuria for both primary analysis and sensitivity analyses.

RAAS is believed to play a crucial role in pathogenesis and progression of DN, involved in glucose metabolism, the regulation of blood pressure, fluid volume and vascular response to injury and inflammation. Its genes consist of renin, angiotensinogen (AGT), angiotensin converting enzyme (ACE), angiotensin converting enzyme 2 (ACE2), angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor (AT2R)[8]. ACE enzyme catalyzes activation of angiotensin I to angiotensin II, resulting in higher intraglomerular pressure and glomerular filtration rate, major contributions to renal damage. ACE I/D polymorphism has been shown to be associated with development and progression of DN, RAAS inhibitors response, declining renal function, and ESRD in type 2 diabetes and type 1 diabetes[4,6,8,9]. The importance of the DD genotype is associated with higher levels of circulating and tissue ACE enzyme activity than ID and II genotypes, which increases risk for DN.

An ethnic effect of ACE I/D polymorphism on the risk for DN has been proposed. Recent studies suggested an association between the ACE I/D polymorphism and the risk for DN development, especially among Asians with type 2 diabetes[6,10,11]. However, there were at least nine Asian studies including ours that did not find statistically higher frequencies of DD genotype and D allele in patients with macroalbuminuria or overt proteinuria. In addition, this polymorphism also showed no association with the development of type 2 diabetes in Thai patients[12]. There are several explanatory factors for inconsistent results, including definition of diabetic nephropathy, differences in case and control definitions, varied genotype distribution (more I allele in studies of Chinese or Japanese population which contrast to our study), and lack of statistical power due to small sample sizes.

One study limitation was that we could not control all risk factors of the macroalbuminuria such as blood pressure and glycemic control since diagnosed diabetes, smoking and ACEI or ARB use. Higher rate of ACEI or ARB usage in our control group may prevent or delay the presence of macroalbuminuria, which may be a confounding factor to true control subjects.

In conclusion, we could not find any association between the I/D polymorphism of the ACE gene and macroalbuminuria in type 2 diabetic Thai patients. However, further investigations with larger sample size or expansion of the scope to other RAAS genes should be performed to confirm the role of genetic factors in developing DN in Thai patients.

What is already known on this topic?

The protective role of II genotype against albuminuria was observed in Caucasians, Indians, Chinese, Japanese and Taiwanese as well as ESRD in Chinese and Koreans. However, there were at least nine Asian studies that did not find statistically higher frequencies of DD genotype and D allele in patients with macroalbuminuria or overt proteinuria studied in Iranian, Bahrain, Turkish, Indians, Japanese, Malaysian and Chinese.

What this study adds?

We demonstrated a higher but not a significantly different frequency of the D allele of the ACE I/D polymorphism in the presence of macroalbuminuria among T2DM Thai patients which agee with some studies from Asian populations.

The test of ACE I/D polymorphism may not be appropriate in T2DM Thai patients as macroalbuminuric predictor or factor in prediction

scores.

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Potential conflicts of interest

None.

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การศึกษาความสัมพันธ์ระหว่างความหลากหลายทางพันธุกรรมของเอนไซม์แองจิโอเท็นซินคอนเวิดติ้งกับภาวะแมคโครอัลบูมินยูเรียของผู้ป่วยโรคเบาหวานชนิดที่สองชาวไทย

ปฏิญัฐ บูรณะทรัพย์ขจร, ณัฐณิชา ห่วงงาม, พิสุทธิ์ กตเวทิน, ธิติ สนับบุญ

วัตถุประสงค์: หาความสัมพันธ์ระหว่างความหลากหลายทางพันธุกรรมของเอนไซม์แองจิโอเท็นซินคอนเวิดติ้งกับภาวะแมคโครอัลบูมินยูเรียของผู้ป่วยโรคเบาหวานชนิดที่สองชาวไทย

วัสดุและวิธีการ: เป็นการวิจัยเชิงวิเคราะห์ ณ จุดเวลาใดเวลาหนึ่งของผู้ป่วยโรคเบาหวานชนิดที่สองชาวไทยจำนวน 188 ราย กลุ่มผู้ป่วยที่เป็นเบาหวานชนิดที่สองเป็นมาอย่างน้อย 10 ปี ไม่มีโปรตีนรั่วในปัสสาวะและระดับครีเอตินินในเลือดไม่เกิน 1.2 มิลลิกรัมต่อเดซิลิตร จัดให้เป็นกลุ่มควบคุมจำนวน 94 ราย ส่วนกลุ่มที่ต้องการศึกษาประกอบด้วยผู้ป่วยโรคเบาหวานชนิดที่สองที่มีภาวะแมคโครอัลบูมินยูเรียจำนวน 94 ราย ทำการเก็บเลือดเพื่อส่งตรวจความหลากหลายทางพันธุกรรมของเอนไซม์แองจิโอเท็นซินคอนเวิดติ้งด้วยวิธีพีซีอาร์

ผลการศึกษา: การกระจายตัวของความหลากหลายทางพันธุกรรมแบบ DD, ID และ II ไม่แตกต่างกันระหว่างกลุ่มผู้ป่วยที่ต้องการศึกษาและกลุ่มควบคุม (II: 20.2%, ID: 40.4%, DD: 39.4%; and II: 25.5%, ID: 43.6%, DD: 30.9%, ตามลำดับ) นอกจากนี้ยังไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของจำนวนไออัลลีล (40.4 และ 47.3%) และ ดีอัลลีล (59.6 และ 52.7%) ระหว่างผู้ป่วยกลุ่มที่ต้องการศึกษาและกลุ่มควบคุมตามลำดับ

สรุป: ความถี่ของดีอัลลีลที่พบมากกว่าในกลุ่มที่ต้องการศึกษาเมื่อเทียบกับกลุ่มควบคุมแต่ไม่มีนัยสำคัญทางสถิติไม่สนับสนุนความสัมพันธ์ของความหลากหลายทางพันธุกรรมของเอนไซม์แองจิโอเท็นซินคอนเวิดติ้งกับภาวะแมคโครอัลบูมินยูเรียในผู้ป่วยเบาหวานชนิดที่สองชาวไทย จำเป็นต้องมีการศึกษาเพิ่มเติมเพื่อหาปัจจัยเสี่ยงทางพันธุกรรมของการเกิดโรคไตจากเบาหวานในผู้ป่วยชาวไทย