Genetics

Angiotensin-I Converting Enzyme Gene Polymorphisms and the Risk of Venous Thromboembolism in an Ethnically Chinese Population Living in Taiwan

Chien-An Hsieh,1 Yu-Lin Ko,2 Tsu-Shiu Hsu,1 Chi-Jen Chang,1 Ming-Sheng Teng,2 Semon Wu2 and Lung-An Hsu1

Background: There have been conflicting reports of the association between the insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene, and the risk of venous thromboembolism (VTE). We sought to investigate the association between ACE I/D polymorphism, and the risk of VTE in a Chinese population living in Taiwan.

Methods: 176 patients with VTE and 321 age and sex-matched controls were analyzed for the ACE I/D polymorphism by polymerase chain reaction.

Results: The genotype distribution of the ACE I/D polymorphism was not statistically different between the VTE affected subjects and the group of unaffected subjects (p = 0.057). Notably, the frequency of ACE D allele in patients with VTE were significantly lower than that in the control group (28% vs. 35%, p = 0.018). After adjusting for age, gender, smoking, hypertension, diabetes and body mass index (BMI), the ACE D allele carriers remained significantly associated with a decreased risk of VTE. Further meta-analysis by pooling data from 15 studies revealed that neither the DD, nor the II genotype, was found to be associated with VTE (pooled unadjusted odds ratio were 1.167, 95% confidence intervals, 0.927-1.470, p = 0.189 for DD, and 1.085, 95% confidence intervals, 0.875-1.345 p = 0.460 for II).

Conclusion: Our results suggest that the presence of the D allele may confer protection against the development of VTE in an ethnically Chinese population in Taiwan. Further meta-analysis did not support a relationship between the ACE I/D polymorphism and the risk of VTE.

Key Words: Angiotensin converting enzyme • Polymorphism • Genetics • Venous thromboembolism

INTRODUCTION

Venous thromboembolism (VTE) is a multifactorial disease influenced by both genetic and environmental factors.1,2 Angiotensin-converting enzyme (ACE) has some biological effects that could be involved in the pathogenesis of VTE, in that ACE converts angiotensin I to angiotensin II, which is a potent vasoconstrictor and can inhibit fibrinolysis, as well as enhance the activation and aggregation of platelets.3 This enzyme also degrades bradykinin, which is a powerful vasodilator and inflammatory mediator.4 Previous studies have suggested that these factors are important in VTE pathogenesis,5,6 thus
implying that the serum ACE level may be associated with the risk of developing VTE.

The ACE gene, located on chromosome 17q23, contains an insertion/deletion (I/D) polymorphism in intron 16, which is characterized by the presence or absence of a 287-bp fragment. This polymorphism accounts for 50% of the inter-individual serum ACE level variation, with the DD genotype reported to have a higher serum ACE level than the ID or II variants.7,8

Several studies previously investigated the relationship between ACE I/D polymorphism and VTE,9-25 but their findings have been controversial. This may have resulted from ethnic differences between study populations. Among them, only one study was comprised of Chinese subjects, and reported an association between the DD genotype and the risk of pulmonary embolism. Therefore, this study attempted to extend and confirm their results for an ethnically Chinese population living in Taiwan.

MATERIALS AND METHODS

Subjects
A total of 186 consecutive and unrelated Taiwanese VTE patients, 87 males and 99 females, were referred to the first cardiovascular division of Chang Gung Memorial Hospital, Lin Kou, Taiwan, for venous Doppler study. Those patients diagnosed with deep vein thrombosis (DVT) and/or pulmonary embolism were enrolled. The mean age of VTE patients was 58.5 ± 15.1 years. Impedance plethysmography and ultrasonographic study confirmed the diagnosis of deep vein thrombosis in all such cases. Of the patients with VTE, 25 had pulmonary embolism. The diagnosis of pulmonary embolism was supported by one of the following confirmatory imaging modalities: ventilation/perfusion radionuclide lung scan, spiral pulmonary computed tomographic scan, or pulmonary angiography. In 40 cases, VTE was associated with cancer (n = 19), pregnancy (n = 5), oral contraceptives (n = 1); or VTE occurred postoperatively (5 cardiac catheterization, 10 surgery). In these cases, VTE was classified as a secondary event. In the other 146 cases, VTE was classified as a primary event. There were 360 control subjects, matched with VTE patients for age (within two years) and sex, randomly selected from among 718 subjects who underwent health examinations, with no known history of major systemic and cardiovascular diseases, including VTE, with mean age of 57.9 ± 14.7 years, including 171 males and 189 females. The demographic details of the VTE patients and their control subjects have been described previously.26 Each subject provided informed consent. The ethics committee at Chang Gung Memorial Hospital approved this study.

Genomic DNA extraction and genotyping of the ACE I/D polymorphism

Genomic DNA of patients and controls was isolated from the peripheral blood leukocytes by standard method using proteinase K digestion of nuclei. The D and I alleles were identified based on polymerase chain reaction amplification (PCR) of the respective segments from intron 16 of the ACE gene, as described previously.27 For quality control purposes, approximately 10% of the samples were re-genotyped using a blinded procedure, with the same results. Further, in order to confirm genotyping, a random sample of subjects was re-genotyped by direct sequence analysis using a commercial sequencing service and the primers previously used for PCR amplification.

Statistical analysis
Clinical characteristics of continuous variables are expressed as means ± standard deviations (SDs), and were tested using a two-sample t test. The chi-square test was used to examine the differences in categorical variables, and to compare the allelic and genotype frequencies. Odds ratios (OR) were calculated as a measure of the association of the ACE I/D genotype with the VTE, with the effects of the D allele assumed to be dominant (which compares a combination of genotypes ID and DD to the homozygous II), recessive (which compares a combination of genotypes ID and II to the homozygous DD), or additive (which compares a combination of genotypes DD and ID with weights 2 and 1 respectively, to the homozygous II). For each odds ratio, we calculated 2-tailed p values and 95% confidence intervals (CI). A multivariable logistic regression analysis was used to evaluate the independent effect of investigated parameters on the risk of VTE. We used Metanalysis software (version 1.2.0, 2004; Technopharma Srl, Italy)
for the meta-analysis. First, the within- and between-study variation or heterogeneity was examined by Cochrane’s Q test. If a significant Q statistic \((p < 0.05)\) indicated heterogeneity across the studies, the DerSimonian and Laird method in the random effects model was used for meta-analysis. Otherwise, the Mantel-Haenszel method in the fixed effect model was selected. Quantification of heterogeneity was also determined by the \(I^2\) metric, which is independent of the number of studies in the meta-analysis. Since not all studies performed and reported the adjusted analysis, and there was also no uniform set of confounders adjusted for by all the studies, we used raw genotype data to yield pooled unadjusted OR (which may be susceptible to confounding). The sample size of our study could determine a relative risk of \(>38\%\) for the D allele being a risk factor for VTE with a power of \(80\%\) at an alpha level of \(0.05\) in a population with an incidence of D allele of \(34\%\).

**RESULTS**

In this study, the genotyping of the ACE I/D polymorphism could not be determined in 10 cases in the case group and 39 cases in the control group because of a shortage of DNA sample. Table 1 lists the baseline characteristics of the cases and control subjects. BMI was significantly higher in VTE patients than in controls. A substantially higher percentage of VTE patients than controls were also diabetics. No significant deviation from Hardy-Weinberg equilibrium was detected for these two polymorphisms in either VTE patients or controls \((p = 0.34, \; 0.62, \; \text{respectively})\). The D allele frequency of the ACE gene in the control group was \(35\%\), and consistent with findings obtained by our previous works. The genotype frequencies in the study samples are shown in Table 2 according to whether the subject has VTE or not. The genotype distribution of the ACE I/D polymorphism was not statistically different between the VTE-affected subjects and the group of unaffected subjects \((p = 0.057)\). In contrast, the ACE D allele frequencies of cases was significantly lower than that in the control group \((28\% \; \text{vs.} \; 35\%, \; p = 0.018, \; \text{Table 2})\). As shown in Table 3, subjects carrying the ACE D allele were significantly associated with a decreased risk of VTE compared with those with homozygous I genotype in dominant and additive models. Although the clinical profiles for the study population were incomplete, the association was also analyzed with a multivariable logistic regression analysis. After adjusting for age, gender, smoking, hypertension, diabetes and BMI, the ACE D allele carriers were significantly associated with a decreased risk of VTE in all studied genetic models (Table 3). Similar results were obtained when cases with pulmonary embolism were compared to controls (data not shown). Moreover, sub-classification of VTE patients into primary or secondary subgroups found no significant difference of the ACE genotype distribution when compared to controls (Table 2). Nevertheless, the ACE D allele carriers were still significantly

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics of study population</th>
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<tbody>
<tr>
<td>Numbers</td>
</tr>
<tr>
<td>Sex (M/F)</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>BMI, kg/m² (n)</td>
</tr>
<tr>
<td>Smoking, % (n)</td>
</tr>
<tr>
<td>Hypertension, % (n)</td>
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<td>Diabetes, % (n)</td>
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</tbody>
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* (n) represented case numbers without missing data; totally, 49 subjects had missing data on BMI status, 26 on hypertension status, 33 on smoking status, and 26 subjects had missing data on diabetes status.

<table>
<thead>
<tr>
<th>Table 2. Genotype and allele frequencies of the ACE I/D polymorphism in the study population</th>
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<tbody>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>ID</td>
</tr>
<tr>
<td>DD</td>
</tr>
<tr>
<td>Allele</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>p</td>
</tr>
<tr>
<td>* All p values are vs. controls.</td>
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</table>
associated with a decreased risk of VTE compared with those with homozygous II genotype in secondary subgroup (Table 2). Figure 1 shows the results of the meta-analysis of our findings, together with 14 previous reports. In total, this meta-analysis comprised 3624 cases and 3812 controls. Cochrane’s Q test was statistically significant (p < 0.001) and $I^2$ was 77%, thus indicating heterogeneity across the studies. Overall, the meta-analysis demonstrated no statistically significant association between the DD genotype and the risk of VTE (DD versus ID + II, the pooled unadjusted OR = 1.167, 95% CI, 0.927-1.470, p = 0.189 by random effect). As shown in Figure 2, similar results were obtained when the effects of the D allele were assumed to be dominant (which compares a combination of genotypes DD and ID to the homozygous II) (pooled unadjusted OR = 1.085, 95% CI 0.857-1.356, p = 0.603 by random effect).

Table 3. Odds ratio (OR) and 95% confidence interval (CI) for venous thromboembolism (VTE) in subjects carrying the ACE I/D polymorphism

<table>
<thead>
<tr>
<th>Model</th>
<th>Genotype</th>
<th>Control VTE</th>
<th>OR (95% CI)</th>
<th>p</th>
<th>OR (95% CI)*</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant</td>
<td>II</td>
<td>138 (43%)</td>
<td>95 (54%)</td>
<td>1.00</td>
<td>0.019</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>ID + DD</td>
<td>183 (57%)</td>
<td>81 (46%)</td>
<td>0.64 (0.44-0.93)</td>
<td>0.58 (0.37-0.89)</td>
<td></td>
</tr>
<tr>
<td>Recessive</td>
<td>II + DD</td>
<td>280 (87.2%)</td>
<td>160 (90.9%)</td>
<td>1.00</td>
<td>0.218</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>DD</td>
<td>41 (12.8%)</td>
<td>16 (9.1%)</td>
<td>0.68 (0.37-1.26)</td>
<td>0.40 (0.18-0.92)</td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td>II</td>
<td>138 (43%)</td>
<td>95 (54%)</td>
<td>1.00</td>
<td>0.02</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>142 (44.2%)</td>
<td>65 (36.9%)</td>
<td>0.72 (0.54-0.95)</td>
<td>0.61 (0.44-0.86)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DD</td>
<td>41 (12.8%)</td>
<td>16 (9.1%)</td>
<td></td>
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</table>

* Multivariable logistic regression analysis with adjustments for age, gender, smoking, hypertension, diabetes and BMI.
95% CI, 0.875-1.345 \( p = 0.460 \); after excluding the study of Della Valle et al.\(^{14} \) in which the numbers of ID and II genotypes were not available). Since we have reported that genetic variants in \( \alpha \) fibrinogen (FGA) gene were associated with susceptibility to VTE in this Taiwanese population,\(^{26} \) we further analyzed a possible gene-gene interaction between the FGA T312A and ACE I/D polymorphisms. We did not find a significant gene-gene interaction (\( p = 0.946 \)) between these two loci on the risk of VTE.

**DISCUSSION**

This study analyzed the association between the ACE gene I/D polymorphism and the susceptibility of VTE in an ethnically Chinese population in Taiwan. Our findings did not reproduce a previous report of association between the ACE DD genotype and VTE on the Chinese in China.\(^{15} \) In contrast, our results suggest that the presence of the D allele may confer protection against the development of VTE. Nevertheless, both Lu’s\(^{15} \) and our studies revealed that the genotype distribution of the ACE I/D polymorphism were not statistically different between the VTE-affected subjects and the group of unaffected subjects. Furthermore, the sample size in this study is substantially larger than that in the study of Lu et al.\(^{15} \)

As shown in Figure 1, several studies have been conducted to investigate the relationship between ACE I/D polymorphism and VTE.\(^{9,25} \) Five of the 14 studies independently demonstrated an association between the ACE DD genotype and increased risk for VTE.\(^{10,13,15,16,19} \) In three studies, a protective effect of the DD genotype against VTE was noted.\(^{11,20,23} \) The remaining six studies did not have statistically significant results. There are several possible reasons for the differences in the findings of the various studies. These include ethnic heterogeneity of the ACE polymorphism, study design, the broad spectrum of patients enrolled, artifacts of small sample size, gene-gene and gene-environmental interaction.\(^{30} \)

Observed association between the ACE D allele and decreased risk of VTE is contrary to the common belief and our initial expectation. Similar results have also been reported in different Caucasian populations.\(^{11,20,23} \)
reasons, however, remain unclear. It could be due to chance fluctuation, or other unknown gene-gene and gene-environmental interactions. VTE is a multifactorial disease influenced by genetic and environmental factors. Primary or idiopathic VTE was defined as a VTE event without any triggering factors such as trauma, surgery, immobilization, pregnancy, or the postpartum period. It is possible that the presence of environmental risk factors alters the association between ACE I/D polymorphism and VTE. Wells et al. postulated that the ACE I/D polymorphism has a protective effect for idiopathic VTE, but this theory is weakened or even reversed if a transient condition is present (such as surgery or cancer). Although our exploratory subgroup analysis was underpowered, we did not find this gene and environmental interaction. Nevertheless, the possibility that ACE I/D polymorphism has differential effects in patients with primary versus secondary VTE still cannot be excluded.

Our study has some limitations. First, the sample size is relatively small. To overcome this limitation on sample size, we incorporated our data into the pooled data from a recent meta-analysis study by Hsiao and Hsu, wherein the combined results still demonstrated no association between VTE and either the DD or the II genotype. Second, there was a lack of information on serum ACE levels. Therefore, whether ACE levels actually contribute directly to VTE cannot be clearly determined. In addition, a positive association between serum ACE levels and VTE has not yet been established. Third, undiagnosed asymptomatic VTE in the controls may be subject to some degree of error. Other limitations included incomplete clinical profiles, and lack of information for use of drugs such as ACE inhibitors and statins. Finally, we did not assess the influence of the ACE I/D polymorphism on the presence of other genetic thrombophilia factors with the exception of FGA gene, we cannot exclude a possible role of gene-gene interaction.

CONCLUSION

In conclusion, our results suggest that the presence of the D allele may confer protection against the development of VTE to a limited extent, but further meta-analysis does not support any association between the ACE I/D polymorphism and the risk of VTE. A larger prospective and longitudinal study, stratifying patients by etiology and plasma ACE activity, would be necessary to fully assess the significance of this polymorphism in the risk of VTE.

REFERENCES


