

ORIGINAL ARTICLE

Association between the insertion/deletion polymorphism of the angiotensin-converting enzyme gene and erectile dysfunction in patients with metabolic syndrome

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This study was designed to investigate whether angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism is associated with erectile dysfunction (ED) in Russian men with metabolic syndrome (MS). A total of 331 men with MS were studied. All patients underwent complex evaluation including the International Index of Erectile Function (IIEF) questionnaire. The ACE I/D polymorphism was determined by polymerase chain reaction. Overall, 182 men (55.0%) had ED according to the IIEF erectile function domain score. In the ED group, the prevalence of DD genotype was found to be significantly higher compared to the non-ED group ($P < 0.001$). In both groups, patients with DD genotype were significantly younger than patients with other genotypes ($P < 0.001$). In addition, in the ED group, the disease affected patients with DD genotype at a significantly younger age ($P < 0.001$). Obtained results give evidence to support the finding that the D allele is a risk factor for the micro- and macrovascular diseases.

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Introduction

The clustering of several cardiovascular disease risk factors—such as disturbances in glucose and insulin metabolism, abdominal obesity, dyslipidemia and hypertension—has been termed the metabolic syndrome (MS).¹ From Reaven's early description of 'syndrome X',² both the prevalence and the general awareness of what is now known as the MS have risen rapidly.³ The diagnosis of the MS appears to identify substantial additional cardiovascular risk above and beyond the individual risk factors.⁴ Therefore, the clinical diagnosis of MS may be a valuable tool for identification of the elusive high-risk patient.

It has now been established that MS has a major association with erectile dysfunction (ED).^{5,6} In a

study comparing 100 men with ED and MS with a control group matched for age and body mass index (BMI), those with the MS had an increased prevalence of ED (26.7 vs 13%, $P = 0.03$). In addition, ED prevalence increased as the number of components of the MS increased.⁵

Although frequently encountered, ED does not affect all men with MS. In this context, it is of interest to elucidate factors determining the increased risk of ED in patients with MS. One of such factors could be the status of the renin–angiotensin–aldosterone system that has long been known to be an important regulator of blood pressure and renal electrolyte homeostasis, and this system has also been implicated in the pathological changes of organ damage in MS through modulation of gene expression, growth, fibrosis and inflammatory response.^{7,8} During detumescence, there is an increase in the level of angiotensin II in cavernous blood and this agent contacted human and canine corpus cavernosum smooth muscle *in vitro*,^{9,10} pointing to the role of the renin–angiotensin–aldosterone in the physiology of erection and pathophysiology of ED.

Angiotensin-converting enzyme (ACE) and angiotensin II activities are under strong genetic

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influence. The existence of a single major genetic influence on circulating ACE levels was first suggested by Cambien and colleagues in 1988.¹¹ Two years later, this genetic influence was shown to be the ACE gene itself: the absence (deletion, D) rather than the presence (insertion, I) of a 287 base pair marker was associated with significantly higher circulating ACE levels.¹²

Since that time a lot of studies were performed in which ACE genotype was used as a tool, through association studies, to investigate the role of tissue ACE in human disease pathogenesis.^{13–17} Taken together, the available evidence supports the notion that the DD ACE genotype adversely influences specific cardiovascular diseases but appears to do so in specific geographical areas and in particular patient subgroups.¹⁸

This can be a possible explanation of the inconsistency of association between ACE genotype and ED. For example, Park *et al.*¹⁹ examined 84 Korean patients with organic ED and 63 control subjects of the same age and found significantly higher incidence of the DD genotype in the first group compared to the second (54 and 24% respectively). On the other hand, Rosas-Vargas *et al.*²⁰ have not found such association in Mexican population.

This study was designed to investigate whether ACE I/D polymorphism is associated with ED in Russian subjects with MS.

Materials and methods

From May 1999 to February 2006, 331 Russian men with MS who consecutively attended the National Center for Prophylactic Medicine were studied. MS was defined using the NCEP/ATP III criteria.²¹ Patients were defined as having MS by the presence of three or more of the following criteria: abdominal obesity; waist circumference >40 inch or BMI \geq 28.8; triglycerides >150 mg/dl; high-density cholesterol <40 mg/dl; blood pressure >130/85 mm Hg; fasting blood glucose >110 mg/dl.

All patients underwent complex evaluation that included detailed sexual, medical and psychological history, International Index of Erectile Function (IIEF) questionnaire, physical examination and laboratory tests (fasting glucose and lipid profile). In all the patients enrolled, an ED diagnosis was made on the basis of the value of the erectile function domain score of the IIEF, according to the classification by Cappelleri *et al.*²²

ACE genotyping was performed by laboratory staff who were unaware of the clinical data. Genomic DNA was extracted from peripheral blood leukocytes. ACE genotypes were determined by the use of polymerase chain reaction (PCR). A set of primers was designed to encompass the polymorphic region in intron 16 of the ACE gene (sense primer CTGGA

GACCACTCCCATCCTTTCT-3' and antisense primer 5'-GATGTGGCCATCACATTCGTCAGAT-3'). After 4 min denaturation at 94°C, DNA was amplified for 35 cycles, each cycle composed of denaturation at 94°C for 60 s, annealing at 59°C for 60 s and extension at 72°C for 60 s. The reaction was terminated at 72°C at 2 min.

The PCR 190-bp fragment for the D allele and 490-bp fragment for I allele were separated by electrophoresis with 2% agarose gel (Tamar) and visualized by ethidium bromide staining.

The study was approved by the Ethics Committee, and a written informed consent was signed by all patients.

Values are expressed as mean \pm s.d. or percentages when appropriate. The statistical difference in genotype distribution and allele frequencies among the patients with and without ED and of categorical variables among genotypes was assessed by the Pearson χ^2 test. ANOVA followed by Scheffe's test was used to compare the group means. All calculated *P*-values were two-sided and *P* < 0.05 was considered statistically significant. All analyses were performed using computer software Statistica 6.0 for Windows.

Results

All 331 patients completed the study protocol and were included in the data analysis. Patient mean age was 48.6 ± 11.0 (range 25–81 years). The prevalence of ED among all patients was 55.0%. ED was scored as mild, mild-to-moderate and severe in 5.5, 41.2 and 53.3% of patients, respectively. In the control group, the mean erectile function domain score of the IIEF was 28.3 ± 1.0 . There was no significant difference between patients with ED (*n* = 182) and without ED (*n* = 149) as far as age, BMI (Table 1), hypertension (93.4 and 92.6%, respectively, *P* = 0.78) and coronary artery disease (CAD) (36.8 and 29.5% respectively, *P* = 0.16) were concerned. Diabetes mellitus was significantly more prevalent in patients with ED (32.4 and 20.1%, respectively, *P* = 0.012).

In the study group, the genotype frequency distributions of ACE polymorphism were in Hardy–Weinberg equilibrium. In the ED group, the prevalence of DD genotype was found to be significantly higher and of II genotype – significantly lower compared to the non-ED group (*P* < 0.001) (Table 1). The majority of patients in both groups carried the D allele, although the patients with ED had somewhat higher proportions of it (81.3 vs 63.8%, *P* < 0.001).

Analysis of interrelationship between ACE polymorphism and age showed that in both groups patients with DD genotype were significantly younger than patients with other genotypes, and patients

Table 1 Clinical characteristics of control subjects and patients with ED divided according to their ACE I/D genotype

| | ED patients | | | | Control subjects | | | |
|------------------------|-------------|-----------|------------|------------------------|------------------|-----------|-----------|-----------------------|
| | Total | II | ID | DD | Total | II | ID | DD |
| N (%) | 182 (100.0) | 10 (5.5)* | 48 (26.4)* | 124 (68.1)* | 149 (100.0) | 15 (10.1) | 78 (52.3) | 56 (37.6) |
| Age (years) | 49.7±9.9 | 54.8±9.0 | 51.5±10.8 | 48.6±9.4 [†] | 47.6±12.3 | 58.1±13.8 | 49.5±14.9 | 42.2±8.3 [‡] |
| BMI | 33.4±4.3 | 33.2±3.0 | 32.4±2.8 | 33.9±4.8 | 33.6±3.1 | 32.4±2.7 | 33.3±3.0 | 34.3±3.4 |
| Age of the onset of ED | 42.8±8.3 | 50.3±9.1 | 47.3±9.4 | 40.4±6.5 [†] | — | — | — | — |
| IIEF EF severity (%) | | | | | | | | |
| Mild (17–25) | 10 (5.5) | 2 (20.0) | 5 (50.0) | 3 (30.0) | — | — | — | — |
| Moderate (11–16) | 75 (41.2) | 6 (8.0) | 35 (46.7) | 34 (45.3) | — | — | — | — |
| Severe (<11) | 97 (53.3) | 2 (2.1) | 8 (8.2) | 87 (89.7) [‡] | — | — | — | — |

Abbreviations: ACE, angiotensin converting enzyme; BMI: body mass index; ED, erectile dysfunction; DD, deletion/deletion; ID, insertion/deletion, IIEF EF: International index of erectile function erectile function domain score.

Data are means±s.d. Comparisons were performed by χ^2 for categorical variables and ANOVA for continuous variables.

* $P<0.001$ vs control group.

[†] $P<0.001$ vs patients from the same group with DD and ID genotype.

[‡] $P<0.001$ vs patients with mild-to-moderate ED.

with DD genotype from the control group were significantly younger than those with the same genotype from ED group ($P<0.001$). In addition, in the ED group the disease affected patients with DD genotype at a significantly younger age ($P<0.001$). At the same time, no differences in age were found between patients with II and ID genotypes from ED and non-ED groups (Table 1).

A significant relationship was determined between ACE polymorphism and ED severity. Patients with severe disease were found to have a higher prevalence of DD genotype compared to men with mild and mild-to-moderate ED ($P<0.001$) (Table 1). The proportion of D-allele carriers also was the highest (93.8%) in patients with severe ED.

Discussion

Our results demonstrate a clear association between ED and ACE genotype in Russian men with MS. Patients with ED had a significantly higher prevalence of ACE DD genotype compared with the control group. Moreover, the DD genotype was also associated with earlier occurrence and more severe forms of the disease.

It is now generally accepted that endothelial dysfunction, defined as an imbalance in which the effects of vasoconstrictors outweigh the effects of vasodilators generally resulting from decreased NO bioactivity,^{23,24} plays a principal role in the development of ED in men with MS.⁵ As an integral component of the renin-angiotensin and kallikrein-kinin systems, ACE promotes synthesis of angiotensin II and degrades bradykinin; therefore, its upregulation may decrease NO activity by reducing bradykinin-mediated release of NO and enhancing angiotensin II-mediated superoxide anion genera-

tion. In such a way, increased ACE activity may contribute to impairments in erectile function.²⁵

Evidence from both *in vivo* and *in vitro* studies supports the role of angiotensin II as an important regulator of tone in penile erectile tissues. *In vitro*, angiotensin II contracted human and canine corpus cavernosum smooth muscle.^{10,25} Elevated levels of angiotensin II have been noted in the systemic and cavernous blood of patients with organic ED, suggesting the role of this peptide in the pathogenesis of ED.⁹ Intracavernosal injection of angiotensin II caused contraction and terminated spontaneous erections in anesthetized dogs, whereas administration of lasortan, selectively blocking angiotensin II receptors, resulted in smooth muscle relaxation and erection.²⁶ The latter agent also improved erectile function and both satisfaction and frequency of sexual activity in clinical settings,²⁷ the finding that further supports the role of angiotensin II in erectile physiology and pathophysiology.

In this study, we confirmed the involvement of renin-angiotensin system in the pathogenesis of ED. The study included men with MS, which had an elevated risk of endothelial dysfunction. Our hypothesis is that the endothelial tissue of such patients is particularly vulnerable to the adverse effects of the increased ACE activity, which is the case in persons with DD genotype. Thus, activation of the renin-angiotensin system precipitates the development of functional impairment of endothelial cells in patients with MS and resulting endothelial function manifests clinically in the form of ED.

An interesting finding of this study was the fact that patients with DD genotype from the control group were significantly younger than those with the same genotype and ED. It is possible that in control patients with ACE DD genotype, by the time of the study, impairments in erectile function have not developed yet.

The prevalence of ED in our patients with MS (55.0%) was considerably higher than that in the study of Esposito *et al.*⁵ (26.7%). One of the possible explanations of this discrepancy could be the difference in mean age (38.4 ± 3.3 vs 48.6 ± 11.0) and BMI (26.9 ± 1.9 vs 33.5 ± 3.6), which were significantly higher in our study.

Although our data need clarification by further observations in other populations, we believe that screening for the ACE I/D polymorphism can become a useful test in the evaluation of men with MS and normal erectile function. Patients with DD genotype that have elevated risk of development of the ED possibly could benefit from more aggressive management focused on the correction of cardiovascular risk factors to prevent and even revert their deleterious effects on the endothelial function. This approach may reduce the risk of ED in this cohort of men. The importance of such strategy is further supported by the fact that ED patients with DD genotype are less likely to respond to phosphodiesterase type-5 inhibitor sildenafil.²⁸ In men with MS, this association could be even more pronounced because of the more severe forms of disease in patients with this genotype, although these assumptions should be further studied.

Conclusions

In summary, we have found an association of the ACE genotype polymorphism with ED in Russian patients with MS. These results give evidence to support the finding that the D allele is a risk factor for the micro- and macrovascular diseases in MS. More studies are required to decide whether the existence of the ACE D allele may be a candidate prediction marker for greater risk of the ED in the rapidly growing population of men with MS.

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