

Association of Angiotensin-Converting Enzyme I/D and eNOS G894T Gene Polymorphisms with Erectile Dysfunction

Angiotensin-Converting Enzim I/D ve ENOS G894T Gen Polimorfizmlerinin Erektile Disfonksiyon ile İlişkisi

F. Sırrı ÇAM, MD,^a
Bilali H. GÜMÜŞ, MD,^b
Ahmet VAR, MD,^c
Afif BERDELİ, MD^d

Departments of

^aMedical Genetics,

^bUrology,

^cBiochemistry,

Celal Bayar University

Faculty of Medicine, Manisa,

^dDepartment of Pediatrics,

Molecular Medicine Laboratory,

Ege University Faculty of Medicine, İzmir

Geliş Tarihi/Received: 25.12.2009

Kabul Tarihi/Accepted: 06.10.2010

Yazışma Adresi/Correspondence:

F. Sırrı ÇAM, MD

Celal Bayar University

Faculty of Medicine,

Department of Medical Genetics, Manisa,

TÜRKİYE/TURKEY

sirri.cam@bayar.edu.tr

ABSTRACT Objective: Recent studies suggest that angiotensin II and nitric oxide (NO) may modulate penile smooth muscle tone and contractility. Because genotypes of the angiotensin converting enzyme (ACE) and endothelial nitric oxide synthase (eNOS) polymorphisms have been associated with disorders in the vascular system, in this study, we investigated an association between the ACE I/D and eNOS G894T gene polymorphisms and erectile dysfunction (ED). **Material and Methods:** A total of 44 patients and 45 control subjects were included in the study. Diagnosis of erectile dysfunction (< 26) was provided by International Index of Erectile Function (IIEF). The ACE I/D ve eNOS G894T gene polymorphisms were genotyped using PCR and RFLP. **Results:** No significant case-control difference was observed for the ACE I/D and eNOS G894T gene polymorphisms either by genotype or allele frequencies [(ACE I/D- $X^2=0.930$ p=0.628) and eNOS 894 G/T- $X^2=2.114$ p=0.348)]. In addition, there was no significant difference between ACE I/D ($X^2=3.174$ p=0.787) and eNOS G894T ($X^2=4.320$ p=0.633) and IIEF scores among the patient group. **Conclusion:** In this study, no association was found between ACE I/D and eNOS G894T gene polymorphisms and erectile dysfunction in the Turkish population studied.

Key Words: Erectile dysfunction; polymorphism, angiotensin-converting enzyme

ÖZET Amaç: Yapılan çalışmalarda nitrik oksit (NO) ve anjiyotensin II'nin penil düz kasların tonüs ve kontraktilesini etkileyebildiği ileri sürülmektedir. Anjiyotensin dönüştürücü enzim (ACE-ADE) ve endotelial nitrik oksit sentaz (eNOS) gen polimorfizmleri damar hastalıkları ile ilişkili olduklarından, bu çalışmada, erektil disfonksiyon ile eNOS 894 G/T (Glu298Asp) ve ACE I/D gen polimorfizmleri arasındaki ilişkinin incelenmesi amaçlanmıştır. **Gereç ve Yöntemler:** Çalışmaya erektil disfonksiyonu (ED) olan 44 hasta ile 45 sağlıklı kontrol grubu alındı. ED tanısı (< 26), Uluslararası Erektile Fonksiyon İndeksine (IIEF) göre değerlendirildi. Hasta ve kontrol grubunda ACE I/D ve eNOS 894 G/T gen polimorfizmleri, polimeraz zincir reaksiyonu ve kısıtlayıcı enzim kesimi yöntemleri ile saptandı. **Bulgular:** ACE I/D ve eNOS 894 G/T gen polimorfizmlerinde hasta ve kontrol gruplarında genotip ve alel frekansı açısından anlamlı bir fark gözlenmedi [(ACE I/D- $X^2=0.930$ p=0.628) ve eNOS 894 G/T- $X^2=2.114$ p=0.348)]. Hasta grubu içerisinde de, IIEF skorları ve ACE I/D ($X^2=3.174$ p=0.787) ve eNOS 894 G/T ($X^2=4.320$ p=0.633) polimorfizmleri açısından anlamlı bir fark bulunmadı. **Sonuç:** Bu çalışmada, çalışılan Türk popülasyonunda ACE I/D ve eNOS 894 G/T gen polimorfizmleri ile ED arasında herhangi bir ilişki olmadığı saptanmıştır.

Anahtar Kelimeler: Sertleşme bozukluğu; çok biçimlilik, angiotensin-converting enzim

Türkiye Klinikleri J Med Sci 2011;31(2):432-7

Erectile dysfunction (ED) is defined as the inability to achieve and maintain an erection sufficient to permit satisfactory sexual intercourse,¹ and is a frequent chronic disorder increasing with age. In a community-based random sample observational study,² the total prevalen-

ce of minimal to severe ED was found as 52%, and the age-adjusted overall prevalence of ED in Turkey was found as 69.2%.³

Normal erectile function is a hemodynamic process, it requires successful integration of many different body systems, including vascular, musculoskeletal, and neurologic systems. The attainment and maintenance of a firm erection requires good arterial inflow of blood as well as efficient trapping of venous outflow and coordination of corporal smooth muscles.^{4,5} Angiotensin converting enzyme (ACE) and nitric oxide (NO) are known to play a role in the regulation of penile vasomotor tone.^{6,7} NO is derived from L-arginine and molecular oxygen, a reaction that is catalyzed by NO synthase (NOS). Three major NOS isoforms are recognized: Ca²⁺-dependent constitutive neuronal (nNOS) and endothelial (eNOS) forms, and a Ca²⁺-independent inducible (iNOS) form.⁸ NO is an important vasoactive molecule and a neurotransmitter essential for relaxation of smooth muscle cells of the corpus cavernosum, and it has important implications for atherogenesis.⁹ The neuronal nitric oxide synthase (nNOS) produces NO in cavernous nerves after parasympathetic activation.¹⁰ Azadzoï et al.¹¹ suggested that iNOS dominated the erectile tissue under ischemic/hypoxic conditions, and increased iNOS levels in vascular tissues might downregulate eNOS and thus inhibit NO production and smooth muscle relaxation. Seftel et al.¹² found that increased iNOS level in diabetic erectile tissue was associated with marked downregulation of eNOS. Another study showed that aging is accompanied.¹³ It is speculated that iNOS played a role in the pathophysiology of ED.

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 generation. In such a way, increased ACE activity may contribute to impairment in erectile function.¹⁴

Erectile dysfunction and cardiovascular disease share the same risk factors such as hypertension, diabetes mellitus, hypercholesterolemia and

smoking.^{15,16} Arterial insufficiency secondary to atherosclerosis, smoking, and trauma have been shown as an important causes of ED.¹⁷ The close relationship between pathogenetic mechanisms in ED and cardiovascular disorders makes an investigation for common genetic factors influencing both disorders feasible.

The gene that encodes eNOS is located on chromosome 7q35 - 36 and consists of 26 exons with a total size of 21 kilobases.¹⁸ The eNOS gene is expressionally and functionally regulated through multiple regulatory steps.^{19,20} Several polymorphisms of this gene have been identified. Among them, the variable number of tandem repeat polymorphism located in intron 4 of eNOS (eNOS4b/a polymorphism) was reported to be significantly associated with development of cardiovascular diseases, and as a smoking-dependent risk for coronary artery disease²¹ and A T-786C variant in the promoter region of the eNOS gene that reduces transcription of the gene is associated with coronary spastic angina and myocardial infarction,²² and Glu298Asp polymorphism (G894T) in exon 7 was reported to be associated with essential hypertension,²³ acute myocardial infarction (MI)^{24,25} coronary artery spasm²⁷ and coronary artery disease.²⁷ The Glu - 298Asp mutation results in an amino acid change. This polymorphism is of particular interest because this conservative amino acid substitution within the oxygenase domain of eNOS may influence eNOS function. In addition, Veldman et. al.,²⁸ reported that the Glu298Asp polymorphism was associated with reduced basal NO production, therefore it seems to be functionally relevant.

The human ACE gene is found on chromosome 17 and a polymorphism consisting of the presence (Insertion, I allele) or the absence (Deletion, D allele) of a 287 - base pair (bp) fragment, has been identified.²⁹ There are three genotypes in this polymorphism: DD and II homozygotes, and ID heterozygote. The II genotype is associated with lower tissue and plasma levels of ACE, whereas the DD genotype is associated with higher ACE levels.³⁰ The D-allele of this polymorphism has been associated with myocardial infarction and left ventricular dysfunction.^{31,32}

Since genotypes of the ACE and eNOS polymorphisms have been associated with disorders in the vascular system in which blood flow regulation is comparable to that in the corpora cavernosa, we investigated the possible correlations among ACE I/D and eNOS G894T gene polymorphisms and ED in a Turkish population.

MATERIAL AND METHODS

ACE I/D ve eNOS G894T gene polymorphisms were analyzed in 44 unrelated Turkish patients with a diagnosis of erectile dysfunction who were admitted to the Department of Urology in Celal Bayar University Hospital, Manisa. Forty five healthy controls were randomly recruited from the same areas with the other cases. Men in the organic erectile dysfunction group were aged between 24-75 years (mean 56 ± 12 years), and between 29-73 years (mean 53 ± 7 years) in the control group. All of them had an active sexual life with a regular partner for ≥ 6 months before the beginning of the study. Patients were evaluated by general medical and family history, sexual history, physical and laboratory examinations. To evaluate the primary organic erectile dysfunction, the patients with diseases such as diabetes mellitus, hypertension, hyperlipidemia, cancer, myocardial infarction, anatomical penile abnormality after spinal cord injury and radical prostatectomy and any medical psychiatric or substance abuse disorders were excluded. All of these men completed the five-item version of the International Index of Erectile Function (IIEF-5) to confirm the diagnosis of ED and evaluate the severity according to the total IIEF-5 score. Those with a score lower than 26 were considered to have ED. The scores 22-25 were classified as mild, 11-21 as moderate, and 1-10 as severe ED. The study was approved by the Ethics Committee of the Celal Bayar University Hospital, and all subjects gave their written informed consent.

MOLECULAR ANALYSIS

Genomic DNA was extracted from 200 μ l of EDTA-anticoagulated peripheral blood leucocytes using the QUIAmp Blood Kit (QIAGEN, Ontario

Canada). eNOS genotyping for the Glu298Asp mutation was performed as described by Hingorani et al.²⁷ The primers used were 5'-CATGAGGC-TCAGCCCCAGAAC-3' (forward) and 5'-AGT-CAATCCCTTTGGTGCTCAC-3' (reverse).²⁷ DNA was amplified for 30 cycles, each cycle comprising denaturation at 95 °C for 1 minute, annealing at 60 °C for 1 minute, extension at 70 °C for 1 minute with final extension time of 5 minutes at 70°C. The initial denaturation stage was carried out at 95°C for 5 minutes. PCR products were digested with the restriction enzyme (MboI) at 37 °C for 16 hours. In the presence of T at nucleotide 894 which corresponded to Asp 298, the 206 base pair (bp) PCR product was cleaved into two fragments of 119 and 87 bp. The PCR products were separated on 2.5% agarose gel and identified by ethidium-bromide staining.

The ACE I/D polymorphism was carried out by polymerase chain reaction (PCR) in a final volume of 15 μ l containing 200 μ M dNTP mix, 1.5 mM $MgCl_2$, 1x Buffer, 1 unit of AmpliTaq polymerase (PE Applied Biosystems) and 10 pmol of each primer. The primers used to encompass the polymorphic region of the ACE were 5'-CTGGAGAC-CACTCCCATCCTTTCT-3' and 5' -ATGTGGCCAT CACATTCGTCAGAT-3'.²⁹ DNA was amplified for 35 cycles, each cycle comprising denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, extension at 72°C for 1 min with final extension time of 7 min. The initial denaturation stage was carried out at 95°C for 5 min. The PCR products were separated on 2.5% agarose gel and identified by ethidium-bromide staining. Each DD genotype was confirmed through a second PCR with primers specific for the insertion sequence.³³

Statistical analysis

Variables are presented as means \pm standart deviation (SD). P-value of 0.05 or less was considered as statistically significant. The frequency of alleles and genotypes in the patient subgroups and normal controls were compared using X^2 test. Statistical analysis was carried out with SPSS for Windows version 11.0 statistical software (SPSS Inc., Chicago, IL, USA).

TABLE 1: ACE- I/D and eNOS 894 G/T genotypes distribution according to the presence of ED.

	Patients (n=44)	Controls (n=45)	
ACE- I/D genotypes			$\chi^2=0.930$ $p=0.628$
II	7 (15.9%)	10 (22.2%)	
ID	24 (54.5%)	25 (55.5%)	
DD	13 (29.6%)	10 (22.3%)	
Allele frequencies I/D	0.432/0.568	0.500/0.500	
eNOS- G/T genotypes			$\chi^2=2.114$ $p=0.348$
GG	23 (52.3%)	30 (66.6%)	
GT	17 (38.6%)	13 (28.9%)	
TT	4 (9.1%)	2 (4.5%)	
Allele frequencies G/T	0.716/0.284	0.811/0.189	

RESULTS

In the study population, in the patients and control groups, mean ages were 56.18 ± 12.14 years, and 53.21 ± 7.8 years, respectively; 43.2% of the patient group were smokers. Distribution of genotype and allele frequencies were similar in erectile dysfunction and control group [ACE I/D (II: 15.9%, ID: 54.5%, DD: 29.6%, in patient group; II: 22.2%, ID: 55.5%, DD: 22.3%, in control group, $\chi^2= 0.930$ $p=0.628$) and eNOS G894T (GG: 52.3%, GT: 38.6%, TT: 9.1%, in patient group; GG: 66.6%, GT: 28.9%, TT: 4.5%, in control group, $\chi^2= 2.114$ $p= 0.348$)] (Table 1). In the evaluation of IIEF, 47.7% of the patient group had severe ED, 38.6% had moderate ED, and 13.7% had mild ED. The populations were in Hardy Weinberg equilibrium. There was no significant difference between ACE I/D ($\chi^2= 3.174$ $p=0.0787$) and eNOS G894T ($\chi^2= 4.320$ $p= 0.633$) and IIEF scores in the patient group (Table 2).

DISCUSSION

In this study, our results failed to show a significant correlation between ED and ACE I/D and eNOS G894T gene polymorphisms in a Turkish population.

To date, there have been few studies on ACE and eNOS gene polymorphisms in ED. ACE may be involved in the pathogenesis of erectile dysfunction because the activation of angiotensin I to an-

giotensin II results in contraction of the penile smooth muscle, and presence and function of angiotensin II, the angiotensin II receptor, and ACE in the corpora cavernosa.^{14,34} Park et al.³⁵ reported that the DD genotype might be seen more frequently in men with a diagnosis of vasculogenic erectile dysfunction in a Korean population. In another study, Mazo et al.³⁶ found that, the prevalence of DD genotype in the ED group was significantly higher than the non-ED group ($p < 0.001$). Another study investigating the same gene polymorphism and ED was published by Eisenhardt et al.⁹ This study enrolled 113 patients with ED and 108 controls. The results showed that patients with ED homozygous for the ACE I allele had a better drug response to sildenafil than did D allele carriers. However, Kim et al.³⁷ did not detect any association between the ACE I/D genotype and ED in the same population, compatible with our results. Rosas-Vargas et al.³⁸ also denied an influence of genotypes of the ACE I/D polymorphism on the risk of ED in a Mexican Mestizo population.

Functional and expression studies suggest that NO plays a key role in the regulation of penile vasodilation in addition to be an important factor for atherogenesis.^{10,39} After the production of endothelial NO by eNOS, NO enhances the production of cGMP by activation of the guanylate cyclase. The cGMP, which induces penile erection has a vasodilating effect on the smooth muscle and the

TABLE 2: ACE- I/D and eNOS 894 G/T genotypes distribution according to the IIEF scores of the patients.

	IIEF scores (n= 44)		
	High	Middle	Mild
ACE- I/D genotypes			
II	4 (9.1%)	3 (6.8%)	-
ID	11 (25.%)	10 (22.7%)	3 (6.8%)
DD	6 (13.7%)	4 (9.1%)	3 (6.8%)
	$\chi^2=2.279$ $p=0.685$		
eNOS- G/T genotypes			
GG	10 (22.7%)	8 (18.2%)	3 (6.8%)
GT	9 (20.5%)	7 (15.9%)	1 (2.3%)
TT	4 (9.1%)	2 (4.5%)	-
	$\chi^2=1.2761$ $p=0.780$		

IIEF: Index of erectile function.

tissue of corpora cavernosa.^{40,41} The data on contribution of the eNOS gene to pathogenesis of ED are conflicting. In a recent study, Lee et al.⁴² suggested that eNOS G894T gene polymorphism might play a role as a genetic susceptibility factor for ED. They found that eNOS 894T allele carriers had significantly greater frequencies of ED (80.0% vs 63.3%; $p=0.04$) and lower IIEF-5 scores (13.2 (5.3) vs. 15.7 (6.1); $p=0.01$) than G allele carriers. In a study from Germany, Eisenhardt et al.⁹ indicated that individuals homozygous for the 894T allele of the eNOS G894T polymorphism showed a reduced response to sildenafil compared with individuals homozygous or heterozygous for the 894G allele. In a Mexican Mestizo population, Rosas-Vargas et al.³⁸ reported that the eNOS 894T allele was associated with an increased risk for ED. In addition, they suggested that this association had a practical implication that might be considered in the development of ED therapy protocols based on the stimulation of penile NO synthesis. In 1999, Park et al.³⁵ performed an investigation on the relationship between eNOS4a/b gene polymorphism and ED in a Korean population. However, their results failed to show a significant correlation between ED and eNOS4a/b gene polymorphism ($p=0.813$). In our community, Erkan et al.⁴³ did not find any association between

ED and eNOS 4a/b gene polymorphism. However, they found that 80% of the patients with severe ED and 54.5% of the diabetic patients with ED had eNOS4a/b genotype, suggesting that diabetic patients with ED tended to have this genotype.

In conclusion, in this study that enrolled 44 patients with ED and 45 controls, no significant difference was observed between ED patients and healthy subjects in the distribution allelic and genotypic frequencies of the ACE I/D and eNOS G894T gene polymorphisms. A small cohort was the limitation of this study. The statistical power increases by increasing the sample size. This means that a large sample has a greater ability than a small sample to detect a clinically important effect if it exists. When the sample size is very small, the test may have an inadequate power to detect a particular effect. It is well known that limitations and complexities exist in "simple" association studies; however, as more data accumulates, a conclusion can be reached. However, more studies with larger subject groups and further molecular studies are needed to clarify the role of the ACE I/D and eNOS G894T gene polymorphism in the development of ED. These might facilitate rational counseling of patients with regard to their individual risk profile and optimal treatment regimens.

REFERENCES

1. NIH Consensus Conference. Impotence. NIH Consensus Development Panel on Impotence. *JAMA* 1993;270(1):83-90.
2. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. *J Urol* 1994;151(1):54-61.
3. Akkus E, Kadioglu A, Esen A, Doran S, Ergen A, Anafarta K, et al.; Turkish Erectile Dysfunction Prevalence Study Group. Prevalence and correlates of erectile dysfunction in Turkey: a population-based study. *Eur Urol* 2002;41(3):298-304.
4. Russell S, Nehra A. The physiology of erectile dysfunction. *Herz* 2003;28(4):277-83.
5. Yurdakul T, Güven S. [Erectile dysfunction physiology and physiopathology]. *Turkiye Klinikleri J Surg Med Sci* 2007;3(38):15-9.
6. Tired L, Rigat B, Visvikis S, Breda C, Corvol P, Cambien F, et al. Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. *Am J Hum Genet* 1992;51(1):197-205.
7. Wattanapitayakul SK, Mihm MJ, Young AP, Bauer JA. Therapeutic implications of human endothelial nitric oxide synthase gene polymorphism. *Trends Pharmacol Sci* 2001;22(7):361-8.
8. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. *Biochem J* 2001;357(Pt 3):593-615.
9. Eisenhardt A, Sperling H, Hauck E, Porst H, Stief C, Rübber H, et al. ACE gene I/D and NOS3 G894T polymorphisms and response to sildenafil in men with erectile dysfunction. *Urology* 2003;62(1):152-7.
10. Andersson KE, Wagner G. Physiology of penile erection. *Physiol Rev* 1995;75(1):191-236.
11. Azadzi KM, Master TA, Siroky MB. Effect of chronic ischemia on constitutive and inducible nitric oxide synthase expression in erectile tissue. *J Androl* 2004;25(3):382-8.
12. Seftel AD, Vaziri ND, Ni Z, Razmjouei K, Fogarty J, Hampel N, et al. Advanced glycation end products in human penis: elevation in diabetic tissue, site of deposition, and possible effect through iNOS or eNOS. *Urology* 1997;50(6):1016-26.
13. Ferrini M, Magee TR, Vernet D, Rajfer J, González-Cadavid NF. Aging-related expression of inducible nitric oxide synthase and markers of tissue damage in the rat penis. *Biol Reprod* 2001;64(3):974-82.
14. Kifer I, Williams GH, Vickers MA, Sullivan MP, Jobbert P, Dluhy RG. Tissue angiotensin II as a modulator of erectile function. I. Angiotensin peptide content, secretion and effects in the corpus cavernosum. *J Urol* 1997;157(5):1920-5.

15. Martin-Morales A, Sanchez-Cruz JJ, Saenz de Tejada I, Rodriguez-Vela L, Jimenez-Cruz JF, Burgos-Rodriguez R. Prevalence and independent risk factors for erectile dysfunction in Spain: results of the Epidemiologia de la Disfuncion Erectil Masculina Study. *J Urol* 2001;166(2):569-74.
16. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. *J Urol* 1994;151(1):54-61.
17. Siroky MB, Azadzi KM. Vasculogenic erectile dysfunction: newer therapeutic strategies. *J Urol* 2003;170(2 Pt 2):S24-9.
18. Marsden PA, Heng HH, Scherer SW, Stewart RJ, Hall AV, Shi XM, et al. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. *J Biol Chem* 1993;268(23):17478-88.
19. Rossi GP, Seccia TM, Nussdorfer GG. Reciprocal regulation of endothelin-1 and nitric oxide: relevance in the physiology and pathology of the cardiovascular system. *Int Rev Cytol* 2001;209:241-72.
20. Andrew PJ, Mayer B. Enzymatic function of nitric oxide synthases. *Cardiovasc Res* 1999; 43(3):521-31.
21. Wang XL, Sim AS, Badenhop RF, McCredie RM, Wilcken DE. A smoking-dependent risk of coronary artery disease associated with a polymorphism of the endothelial nitric oxide synthase gene. *Nat Med* 1996;2(1):41-5.
22. Nakayama T, Soma M, Takahashi Y, Izumi Y, Kanmatsuse K, Esumi M. Association analysis of CA repeat polymorphism of the endothelial nitric oxide synthase gene with essential hypertension in Japanese. *Clin Genet* 1997; 51(1):26-30.
23. Miyamoto Y, Saito Y, Kajiyama N, Yoshimura M, Shimasaki Y, Nakayama M, et al. Endothelial nitric oxide synthase gene is positively associated with essential hypertension. *Hypertension* 1998;32(1):3-8.
24. Shimasaki Y, Yasue H, Yoshimura M, Nakayama M, Kugiyama K, Ogawa H, et al. Association of the missense Glu298Asp variant of the endothelial nitric oxide synthase gene with myocardial infarction. *J Am Coll Cardiol* 1998;31(7):1506-10.
25. Hibi K, Ishigami T, Tamura K, Mizushima S, Nyui N, Fujita T, et al. Endothelial nitric oxide synthase gene polymorphism and acute myocardial infarction. *Hypertension* 1998;32(3): 521-6.
26. Yoshimura M, Yasue H, Nakayama M, Shimasaki Y, Sumida H, Sugiyama S, et al. A missense Glu298Asp variant in the endothelial nitric oxide synthase gene is associated with coronary spasm in the Japanese. *Hum Genet* 1998;103(1):65-9.
27. Hingorani AD, Liang CF, Fatibene J, Lyon A, Monteith S, Parsons A, et al. A common variant of the endothelial nitric oxide synthase (Glu298-->Asp) is a major risk factor for coronary artery disease in the UK. *Circulation* 1999;100(14):1515-20.
28. Veldman BA, Spiering W, Doevendans PA, Vervoort G, Kroon AA, de Leeuw PW, et al. The Glu298Asp polymorphism of the NOS 3 gene as a determinant of the baseline production of nitric oxide. *J Hypertens* 2002; 20(10):2023-7.
29. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). *Nucleic Acids Res* 1992;20(6):1433.
30. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990;86(4):1343-6.
31. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992;359(6396): 641-4.
32. Marian AJ, Yu QT, Workman R, Greve G, Roberts R. Angiotensin-converting enzyme polymorphism in hypertrophic cardiomyopathy and sudden cardiac death. *Lancet* 1993; 342(8879):1085-6.
33. Shanmugam V, Sell KW, Saha BK. Mistyping ACE heterozygotes. *PCR Methods Appl* 1993;3(2):120-1.
34. Park JK, Kim SZ, Kim SH, Park YK, Cho KW. Renin angiotensin system in rabbit corpus cavernosum: functional characterization of angiotensin II receptors. *J Urol* 1997;158(2): 653-8.
35. Park JK, Kim W, Kim SW, Koh GY, Park SK. Gene-polymorphisms of angiotensin converting enzyme and endothelial nitric oxide synthase in patients with erectile dysfunction. *Int J Impot Res* 1999;11(5):273-6.
36. Mazo EB, Gamidov SI, Mamedov MN, Iremashvili VV. Association between the insertion/deletion polymorphism of the angiotensin-converting enzyme gene and erectile dysfunction in patients with metabolic syndrome. *Int J Impot Res* 2008;20(1):68-72.
37. Kim DS, Choi SI, Lee HS, Park JK, Yi HK. Determination of human angiotensin converting enzyme (ACE) gene polymorphisms in erectile dysfunction: frequency differences of ACE gene polymorphisms according to the method of analysis. *Clin Chem Lab Med* 2001;39(1): 11-4.
38. Rosas-Vargas H, Coral-Vazquez RM, Tapia R, Borja JL, Salas RA, Salamanca F. Glu298Asp endothelial nitric oxide synthase polymorphism is a risk factor for erectile dysfunction in the Mexican Mestizo population. *J Androl* 2004;25(5):728-32.
39. Schmidt HH, Walter U. NO at work. *Cell* 1994;78(6):919-25.
40. Bloch W, Klotz T, Sedlaczek P, Zumbé J, Engelmann U, Addicks K. Evidence for the involvement of endothelial nitric oxide synthase from smooth muscle cells in the erectile function of the human corpus cavernosum. *Urol Res* 1998;26(2):129-35.
41. Andersson KE. Pharmacology of penile erection. *Pharmacol Rev* 2001;53(3):417-50.
42. Lee YC, Huang CH, Wang CJ, Liu CC, Wu WJ, Chang LL, et al. The associations among eNOS G894T gene polymorphism, erectile dysfunction and related risk factors. *BJU Int* 2007;100(5):1116-20.
43. Erkan E, Muslumanoglu AY, Oktar T, Sanli O, Ozbek U, Kadioglu A. Polymorphism of endothelial nitric oxide synthase gene in patients with erectile dysfunction. *J Sex Med* 2006; 3(1):69-75.