ACE Gene Insertion/Deletion Polymorphism Seminal Associations in Infertile Men

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Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
AT1 = angiotensin II receptor type I
D = deletion
HOS = hypo-osmotic swelling
I = insertion
OAT = oligoasthenoteratozoospermia
PCR = polymerase chain reaction
$PGF_{2\alpha}$ = prostaglandin-$F_{2\alpha}$
ROS = reactive oxygen species
sACE = somatic ACE
TAC = total antioxidant capacity
tACE = testis specific ACE

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Purpose: We assessed seminal associations of the ACE* gene insertion/deletion polymorphism in infertile men.

Materials and Methods: A total of 405 men were investigated, divided into healthy fertile men, and those with asthenozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia, respectively. They underwent semen analysis, and assessment of sperm acrosin activity, hypo-osmotic swelling, seminal 8-iso-prostaglandin-$F_{2\alpha}$, total antioxidant capacity, α-glucosidase and ACE gene polymorphisms.

Result: The ACE* insertion/insertion genotype was noted in 182 men, including 76.5% of healthy fertile men, and 47.4%, 39.8% and 17.6% of those with asthenozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia, respectively. The ACE* insertion/deletion genotype was noted in 133 men, including 13.7% of healthy fertile men, and 42.3%, 27.5% and 47.2% of those with asthenozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia, respectively. The ACE* deletion/deletion genotype was identified in 90 men, including 9.8% of healthy fertile men, 10.3%, 32.70% and 35.2% of those with asthenozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia, respectively. Men with the ACE* deletion/deletion and insertion/deletion genotypes showed a significant decrease in sperm count, motility, linear velocity and normal forms, acrosin activity index, hypo-osmotic swelling test and seminal α-glucosidase, and significantly increased seminal 8-iso-prostaglandin-$F_{2\alpha}$ than those with the ACE* insertion/insertion genotype.

Conclusions: ACE gene deletion polymorphism is associated with abnormal seminal variables, such that carriers of the ACE* deletion/deletion genotype have higher seminal oxidative stress.

Key Words: testis; infertility, male; spermatozoa; peptidyl-dipeptidase A; oxidative stress

Angiotensin converting enzyme is a membrane bound, dipeptidyl carboxypeptidase that catalyzes the conversion of angiotensin I to the physiologically active octapeptide angiotensin II. Angiotensin II is one of the important components in the renin-angiotensin system that controls fluid-electrolyte balance and blood pressure.[1]

The human ACE gene is located on chromosome 17 at q23. It includes 26 exons and 25 introns. Two isoforms of ACE exist in mammals, of which sACE, the larger one, is expressed in blood, and vascular endothelial, renal epithelial and testicular Leydig cells. The testis specific form tACE is expressed only in developing male germ

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cells. They differ in that sACE has 2 active sites (N and C-termini) while tACE has a single active site analogous to the C-terminal portion of sACE.[2]

Serum and tissue ACE activity are usually associated with a common ACE gene variant. The insertion/deletion ACE* I/D polymorphism of the ACE gene is characterized by I or D of a 287 bp Alu repeat sequence in intron 16 of the gene.[3] Mean ACE activity in ACE* D/D carriers is twice that in ACE* I/I genotype individuals while those with the ACE* I/D genotype have intermediate levels, indicating codominancy. The ACE* I/D polymorphism accounted for 47% of the observed variance in ACE and is associated with differences in ACE levels in various tissues and organs, including the testis.[4]

Barley et al suggested that ethnic origin should be carefully considered in the increasing number of studies of the ACE* I/D genotype and disease etiology.[5] Experimentally male mice lacking tACE had decreased fertility due to a defect in sperm migration in the oviducts and decreased ability of spermatozoa to bind to the zona pellucidae.[6] Kessler et al reported that male fertility in ACE null mice was restored by the expression of tACE but not sACE in spermatozoa,[7] suggesting that molecular defects in the tACE gene could have a role in male infertility.

Since angiotensin II mediates its effects through AT1 and spermatozoa have a functional AT1 receptor that controls sperm motility, AT1 receptor would be expected to potentiate oxidative stress and promote tissue damage through membrane bound NADPH oxidase activity, which is a major source of ROS generation.[8] Thus, ACE gene polymorphism was proposed to have an impact on spermatozoa, which are susceptible to ROS induced damage since their plasma membranes contain a lot of polyunsaturated fatty acids.[9]

We investigated the seminal association of the ACE* I/D gene polymorphism in infertile men.

MATERIALS AND METHODS

We investigated 405 men of white ethnic origin who were recruited from the andrology department at University Hospital after we received institutional review board approval and informed consent. They were divided into 4 groups, including 102 healthy fertile men, 97 with asthenozoospermia, 98 with asthenoteratozoospermia and 108 with OAT.

Healthy fertile men had normozoospermic semen analysis and had fathered a child within the previous 2 years. The other investigated groups with primary male factor infertility were classified by semen analysis based on WHO criteria.[10] Study exclusion criteria were leukocytospermia, hypertension, diabetes, cryptorchidism, varicocele, abnormal karyotyping and Y chromosome deletions. Participants provided a history and underwent clinical examination, semen analysis, HOS test, ACE gene polymorphism analysis, and assessment of sperm acrosin activity, seminal 8-iso-$PGF_{2\alpha}$, TAC and α-glucosidase.

Semen samples were left for 30 minutes for liquefaction and then assessed by WHO guidelines.[10] Sperm morphology was evaluated by phase contrast microscopy and sperm Mac stain (FertiPro, Beerneme, Belgium). Spermatozoa were separated by Sil-Select gradient (FertiPro). Purified spermatozoa were used to assess acrosin activity and membrane integrity with the HOS test. Seminal plasma was obtained by centrifuging the semen sample at 4,000 $\times$ gravity for 15 minutes to assess seminal 8-iso-$PGF_{2\alpha}$, TAC and α-glucosidase activity.

Also, a whole blood sample was collected in tubes containing ethylenediaminetetraacetic acid for DNA extraction and ACE gene polymorphism evaluation. Genomic DNA was extracted from peripheral blood using a genomic DNA purification kit (Genta, Berkley Heights, New Jersey). The ACE* I/D genotype polymorphism was identified by detecting the Alu repetition sequence of 287 bp intron 16 with PCR.[11] The sense oligonucleotide primer was 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and the antisense primer was 5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′. These primers allowed the detection of a genomic DNA segment of 490 bp, corresponding to the I allele, and a segment of 190 bp, corresponding to the D allele. ACE genotypes were classified as I/I, I/D or D/D depending on whether each allele had this sequence.

The reactions were performed in a final volume of 50 μl containing 25 pmole of each primer, 1.5 mmol/l $MgCl_2$, 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.4), 0.1 mg/ml gelatin, 0.2 mmol/l of each dideoxynucleotide triphosphate and 1.25 U Taq DNA polymerase (Gibco®). The amplification profile included initial denaturation at 95C for 5 minutes, 35 cycles of denaturation at 95C for 1 minute, annealing at 58C for 1 minute and extension at 72C for 2 minutes, followed by final extension at 72C for 10 minutes. PCR products were electrophoresed on 2% agarose gel and DNA visualized with ethidium bromide staining. ACE* I/D polymorphism revealed 1 of 3 electrophoretic patterns, including the ACE* I/I genotype with a fragment at 490 bp, ACE* D/D genotype with a fragment at 190 bp, and ACE* I/D genotype with 2 fragments at 490 and 190 bp, respectively (fig. 1).

For the HOS test[12] 1 ml freshly prepared hypo-osmotic medium composed of 0.735 gm $Na_3C_6H_5O_7{\cdot}2H_2O$ and 1.351 gm fructose in 100 ml distilled water was mixed with 0.1 ml liquefied semen. This was incubated at 37C for 30 minutes. Spermatozoa were examined under phase contrast microscopy. Sperm tail swelling was identified and counted in duplicate in 100 spermatozoa.

Sperm acrosin activity[13] was assessed by gelatinolysis. Gelatin covered slides were prepared by spreading 20 μl 5% gelatin (Merck, Darmstadt, Germany) in distilled water. Slides were air dried, stored at 4C overnight, fixed and washed in phosphate buffered saline. Purified spermatozoa were diluted 1:10 in phosphate buffered saline containing 15.7 mM α-D-glucose. Semen samples were smeared on prepared slides and incubated in a moist chamber at 37C for 2 hours. The halo diameter around any 10 spermatozoa was measured in phase contrast with an eyepiece micrometer. The halo formation rate was calculated per slide as the percent of spermatozoa showing a