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GENETICS

The prevalence of common CFTR mutations in Iranian infertile men with non-CAVD obstructive azoospermia by using ARMS PCR techniques

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Abstract
Purpose To evaluate five common cystic fibrosis transmembrane conductance regulator (CFTR) mutations (ΔF508, G542X, R117H, W1282X and N1303K) in the Iranian infertile men with noncongenital absence of vas deferens (CAVD) obstructive azoospermia.
Methods The common CFTR gene mutations were tested on blood samples from 53 infertile men with non-CAVD obstructive azoospermia and 50 normal men as control individuals. Genomic DNA is extracted from the whole blood and the common CFTR mutations have been detected by the amplification refractory mutation system (ARMS) techniques.
Results The common CFTR mutations were found positive in 5/53)9.43%(for ΔF508 and 4/53)7.55%(for G542X mutation of all patients tested. Also, no CFTR mutations were detected in the normal men.
Conclusion The common CFTR mutations were detected in 9/53(17%) infertile men with non-CAVD obstructive azoospermia. Pre-treatment CFTR mutation analysis remains critical to distinguish cystic fibrosis (CF) genotypes for men with non CAVD obstructive azoospermia.

Keywords Obstructive azoospermia · Common CFTR gene mutations · Non CAVD · Infertile men · ARMS PCR

Capsule The common CFTR mutations were detected in 9/53 infertile men with non- CAVD obstructive azoospermia by using ARMS techniques. Pre-treatment CFTR mutation analysis stands critical in these patients.

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Introduction

Infertility is defined as the inability to conceive after 1 year of regular unprotected sexual intercourse. About 15% of couples are affected. Male factors account for about 50% of couples with infertility [1]. Approximately 10% to 15% of infertile men suffer from azoospermia complete absence of sperms during the ejaculation. Azoospermia results from obstruction of extra testicular ducts (obstructive) or testicular dysfunction (non-obstructive) [2, 3] .Among these azoospermic patients, approximately 40% of them have complete obstruction in the ductal system and hence suffer from obstructive azoospermia [4]. Obstructive azoospermia, due to an anatomical block in either the epididymis or the vas deferens, is one of the surgically correctable causes of male infertility and is thus associated with a good outcome[5].

Many genes are likely to be involved in the complex process of reproduction[6].Infertility, or at least subfertility, in men with CF was first suspected in the 1960s[7]. Infertility in men with CF typically originates in developmental abnormalities in the vas deferens or the distal half of the epididymis. In 97–98% of men with CF, a congenital bilateral absence of the vas deferens (CBAVD) blocks the transport of spermatozoa from testicular or epididymal structures to the outer genital tract, resulting in azoospermia [8]. There are reports that CFTR is also involved in non CAVD obstructive azoospermia [9–13]. In a small study of 17 Patients having obstructive azoospermia in which the vas deferens and/or epididymis is present but obstructed, a mutation in the CFTR gene was identified in eight of 34(23.5%) [9].

The CFTR gene spans about 190 kb at the genomic level and contains 27exons. The CFTR protein is a glycosylated transmembrane protein, which functions as a chloride channel. CFTR is expressed in epithelial cells of exocrine tissues, such as the lungs, pancreas, sweat glands and vas deferens [14].

There are more than 1,800 mutations of CFTR and most of them may not cause typical CF phenotypes, but may lead to compromised spermatogenesis [15].

CFTR mutations with impaired CFTR function may lead to reduced sperm fertilizing capacity and male infertility other than CBAVD [16].

Due to the high incidence of CFTR mutations in patients with obstructive azoospermia, screening of CFTR mutations before assisted reproduction is recommended [17].

This gene is located on the short arm of chromosome 7 and encodes the same membrane protein that, apart from its chloride channel function, also influences the formation of the ejaculatory duct, seminal vesicle, vas deferens and distal two thirds of the epididymis [18].

The ARMS system has several advantages over other PCR-based analysis systems. The method is rapid, reliable, and nonisotopic, and results can be easily obtained in one working day. The use of two reactions with internal controls ensures that false-negative results are not obtained.

The aim of this study was to evaluate five common CF mutations (ΔF508, G542X, R117H,W1282X, N1303K)by use of the multiplex and single ARMS system among Iranian men with non-CAVD obstructive azoospermia (including those with idiopathic epididymal or ejaculatory duct obstruction) as the first descriptive study.

Materials and methods

This study is being conducted at the Department of Genetic in Special Medical Center, Tehran, Iran over the years 2010–2011. Blood samples were collected from 53 males with non CAVD obstructive azoospermia in the Royan Infertility Center in Qom, Iran and from 50 normal men (men with normal fertility and sperm parameters) who were employees and workers of the Royan Infertility Center and Khorrami hospital in Qom, Iran.

All the patients and control individuals were informed and prepared written consent to the procedures of the study. The diagnosis of non-CAVD obstructive azoospermia is based on the following examinations: normal semen volume; normal testicular size; presence of the vas deferens by clinical examination; normal levels of serum follicle-stimulating hormone (FSH);azoospermia; absence or low levels of fructose and presence of spermatozoa in sample extracted by percutaneous sperm aspiration(PESA).No other symptoms of CF such as chronic lung inflammation/infection, pancreatic

Table 1 Allelic and Genotypic Frequencies in Iranian infertile men with non-CAVD obstructive azoospermia

Mutation	No. of chromosomes carry CF allele	%(Allelic frequencies)	Genotype	No. of patients	%(Genotypic frequencies)
ΔF508	5/106	4.7	ΔF508/+	5	9.43
G542X	4/106	3.77	G542X/+	4	7.55
R117H	0/106	0	R117H/+	0	0
W1282X	0/106	0	W1282X/+	0	0
N1303K	0/106	0	N1303K/+	0	0
Normal	97/106	91.5	+/+	44	83
Total	106/106	100.00	Total	53	100.00

insufficiency and intestinal obstruction have been reported in clinical file of our patients.

Genomic DNA is extracted from the whole blood by a non-organic method involving lysis, proteinase K digestion and salting out of DNA with isopropanol precipitation [19, 20]. All DNA samples were analyzed, using the primer sequence and single and multiplex ARMS-PCR technique as described by Ferrie et al. [21], for the following mutations: ΔF508, N1303K, G542X,W1282X,R117H mutations.W1282X and R117H mutations were analyzed by single ARMS-PCR technique and ΔF508, N1303K and G542X mutations were analyzed simultaneously by multiplex ARMS-PCR technique. ARMS PCR program for ΔF508, N1303K, G542X,R117H began with a 5 min incubation at 94°C,andProceeded with 28 cycles, each containing 15 s of denaturation at 94°C,30 s of annealing at appropriate temperature and 30 s of extension at 72°C;with a 10 min incubation at 72°C completing the amplification. ARMS PCR program for W1282X mutation began with a 1 min incubation at 95°C, and Proceeded with 32 cycles, each with 1 min of denaturation at 95°C, 30 s of annealing at appropriate temperature and 45 s of extension at 72°C; with a 5 min incubation at 72°C completing the amplification.PCR conditions for amplification of above DNA samples stood as described earlier [21].

Results

Heterozygote frequency for ΔF508 mutation has been 5/53 (%9.43) and for G542X mutation, it has been 4/53(%7.55) in all patients tested where as other common mutations (R117H, W1282X, N1303K) were not detected in our samples. Also, no common CFTR mutations were detected in all normal men. In other words, frequency for ΔF508 mutation was 5/106 (%4.7) and for G542X mutation it proved 4/106(3.77) in all chromosomes tested.

Table 1 summarizes the frequencies of those mutations and heterozygots.

Discussion

CF is one of the most common autosomal recessive diseases in Caucasians caused by defects in CFTR gene. CF is fatal and has been reported in many populations. Also, formation of the ejaculatory duct, seminal vesicle, vasdeferens and distal two thirds of the epididymis is affected by this gene [18]. Many studies about correlation between CFTR mutations and CAVD have been previously reported [22–27].

There are reports that CFTR is also involved in non-CAVD obstructive azoospermia (other forms of obstructive azoospermia than CAVD)[9–13].For example, in a small study of 17 patients having obstructive azoospermia in which the vas deferens and/or epididymis is present but obstructed, a mutation in the CFTR gene has been identified in the eight of 34 CFTR genes (23.5%).

Among 53 patients with non-CAVD obstructive azoospermia, five were heterozygotes for ΔF508 mutation (9.43%), and four patients carried G542X mutation (7.55%) whereas other mutations (N1303K, W1282X andR117H) were not detected in our samples.

Selection of our sample size occurred based on statistician advice and review of other studies. Also, choice of five mutations is based on high frequency of these mutations reported in CFTR gene and some of them prevailing in patients with obstructive azoospermia.

Our study is consistent with studies that have been previously reported [9–13] and is not consistent with some of previous studies [28].These differences may be due to the clinical criteria applied when selecting the patients, race and sample size of patients.

Also ARMS PCR technique (single or multiplex) is cheap, precise and very rapid technique for mutation analysis; therefore, in clinical center such as Infertility Center where diagnosis should be carried out rapidly, ARMS PCR technique at least for detection of common CFTR mutations is a preferable technique.

In summary, our data provide further evidence of a strong association between infertile men with non-CAVD obstructive azoospermia and CFTR mutations.

The identification of mutations in these patients provides important information for the genetic counseling of couples prior to infertility treatment. Because of correlation between non-CAVD obstructive azoospermia and CFTR mutations as shown by recent and previous studies [9–13], it is recommended that, before infertility treatment in these patients who stand by genetic counseling and following CFTR mutations (at least common CFTR mutations), screening be carried out for prevention of cystic fibrosis in the next generation; if male partner proves positive for CFTR mutation, female partner also should be tested before infertility treatment.

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