A Survey of the Common Mutations and IVS8-Tn Polymorphism of Cystic Fibrosis Transmembrane Conductance Regulator Gene in Infertile Men with Nonobstructive Azoospermia and CBAVD in Iranian Population

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ABSTRACT

Background: Studies have revealed a strong association between mutations of CFTR gene and the congenital bilateral absence of the vas deferens (CBAVD), but the role of this gene in other types of male infertility is still unclear. The purpose of this study was to investigate the frequency of the most common mutations of the CFTR gene (ΔF508, G542X, N1303K, G551D, and W1282X) in a population of infertile men with nonobstructive azoospermia (NOA) and CBAVD in Iran. Methods: Blood samples were obtained from 50 NOA, 50 CBAVD, and 100 normal males (control). Genomic DNA was isolated from whole blood leukocytes, and the presence of common mutations of the CFTR gene was assessed by an amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). Restriction fragment length polymorphism (PCR-RFLP) was also used to analyze IVS8-Tn polymorphism. Results: It was found that 16%, 8%, and 8% of patients with CBAVD were heterozygote for ΔF508, G542X, and N1303K, respectively. The frequency of the 5T allele was 34% and higher than the normal group (p < 0.001). None of the common CFTR gene mutations were detected in NOA patients, and no significant difference was found in the distribution of the 5T allele between the NOA patients and the control group (5 vs. 3 p = 0.721). Conclusion: Based on the present case-control study, the CFTR gene mutations and IVS8-Tn polymorphisms are correlated with CBAVD; however, extensive investigations are necessary to determine the exact relationship between the gene mutations and other forms of male infertility. DOI: 10.29252/ibj.23.2.92

Keywords: Azoospermia, CFTR, Congenital bilateral absence of the vas deferens, Male Infertility, Mutation

INTRODUCTION

Infertility is commonly known as the inability to become pregnant after one year of frequent intercourse, in the absence of a contraceptive[1]. Male infertility is a major health problem in the global populations; its prevalence in Western countries has been estimated to be about 20%[2]. The main causes of male factor infertility are azoospermia, asthenozoospermia, teratozoospermia, and oligozoospermia. Azoospermia is characterized as the lack of sperms in the ejaculate, and it can be divided into two groups: nonobstructive azoospermia (NOA) and obstructive azoospermia (OA). The former is usually due to spermatogenesis failure, while the latter is caused by an obstruction in the seminal tract (epididymis, vas deferens, and ejaculatory ducts)[3-5]. The congenital bilateral absence of the vas deferens...
(CBAVD) accounted for 25% of cases of OA and 1.5% of male infertility[6,7].

It has been estimated that more than 3000 genes are involved in the genetic regulation of male or female fertility[8-10]. The CFTR gene is located on chromosome 7 (7q31.2) and contains 27 exons that span 190 kb of genomic DNA. It encodes a chloride channel in epithelial cells. Mutations in this gene result in cystic fibrosis (CF). The clinical manifestations of CF include chronic obstructive pulmonary disease, pancreatic insufficiency, high levels of sweat electrolytes, and male infertility[11,13]. In most cases, CBAVD is regarded as a genital form of CF, without other clinical manifestations[13]. More than 2000 mutations of the CFTR gene have been found, which has led to a variety of clinical phenotypes of CF[14]. \( \Delta F508, N1303K, \) and \( G542X \) have been identified as the most frequent CF-causing mutations worldwide. Several studies have reported the role of CFTR gene mutations in CBAVD in men and congenital unilateral absence of the vas deferens[11,15,17]. The CFTR gene mutations associated with this disorder are as follows: \( \Delta F508, R117H, G551D, D1152H, G542X, M470W, R334W, R74W, M9521, W1282X, N1303K, \) and \( G85E \)[18-20]. Based on the phenotypic effects, there are usually two types of alleles in the CF genotype: (1) severe allele and (2) mild allele. \( \Delta F508 \) and \( G542X \) are characterized as severe alleles, while \( R117H \) is regarded as a mild allele. Numerous reports have indicated that CFTR gene can be involved in another cases of infertility other than CBAVD[21-23]. However, it is not known for sure whether these mutations have an important function in the spermatogenesis and NOA.

The present study aimed to evaluate the frequency of common mutations of the CFTR gene including, \( \Delta F508, G551D, G542X, N1303K, \) and \( W1282X \) in Iranian infertile men with NOA and CBAVD using ARM-PCR technique. Also, IVS8-Tn polymorphism was analyzed by RFLP-PCR.

**MATERIALS AND METHODS**

This case-control study was conducted on 50 CBAVD patients, 50 NOA study, and a control group (n = 100), who had at least one child; the patients came from Yazd Infertility Center (Yazd) and Mirza Koochak Khan Hospital (Tehran, Iran). The study was conducted at the Medical Genetics Department of the National Institute of Genetic Engineering and Biotechnology (NIGEB, Tehran). The diagnosis of CBAVD was initially suggested by palpable scrotal vas deferens on physical examination and transabdominal/rectal ultrasonography and then confirmed by cytobiochemical characteristics, according to the World Health Organization criteria[24,25]. The diagnosis of NOA was based on the following examinations: normal semen volume, normal testicular size, presence of the vas deferens by clinical examination, and normal levels of serum follicle-stimulating hormone and also according to the medical history of patients; none of them had chromosomal aberrations or Y-chromosome microdeletions. No other symptoms of CF such as chronic lung inflammation/ infection, pancreatic insufficiency, and intestinal obstruction were observed in these patients. All subjects gave their written informed consent, and then 5 mL of whole blood sample was collected in EDTA vacuum tubes (Becton Dickinson, USA). Genomic DNA was extracted from peripheral blood leukocyte by salting-out method and was analyzed for the most common CF mutations, including \( \Delta F508, G551D, G542X, N1303K, \) and \( W1282X \) using amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method as described previously[26]. IVS8-Tn polymorphism was analyzed by using restriction fragment length polymorphism (RFLP)-PCR. The CF intron 8/exon 9 (product size: 259-261 bp) primers were as follows: common downstream primer, CF9RR: GACATGGACACCA AATTAAG; upstream primer, CF5T: TGTGTGTGTGTGTGTGTGTGT G*TT), and upstream primer, CF7T: GTGTGT GTGTGTGTGTGTGTGGTT; *denotes a mismatch. Amplification reaction was performed using a conventional protocol: the reaction mixture contained 10 mM of Tris-HCl (pH 8.3), 50 mM of KCl, 1.5 mM of MgCl\( _2 \), 0.01 mg/mL of gelatin, 0.2 mM of each deoxynucleotide, and 0.75 mM of each primer and 1 U of AmpliTaq Polymerase in a total volume of 25 \( \mu \)L. The PCR thermal cycles were: 94 °C for 5 min, followed by 32 cycles of 1 min at 94 °C, 1 min at 58 °C (annealing), 1 min at 72 °C (extension), and a final extension of 5 min at 72 °C. Twenty microliters of the amplified product of 260 to 264 bp (intron 8) was digested with 5 to 10 U HpaI for 3 h or at 37 °C overnight. After digestion with HpaI, the products were run on an 8% acrylamide gel with 0.5x Tris-borate-ethylenediaminetetraacetic acid at 200 V for 3 h (Fig. 1). The expected product sizes of different digests are listed in Table 1[27]. The differences between both groups (patients and control) were analyzed using SPSS for Windows software (version 18.0). All \( p \) values were based on two-sided comparisons. \( p \) values less than 0.05 were considered to indicate statistical significance.
Mutation analysis was performed on all the 100 patients (NOA and CBAVD patients) and 100 fertile controls. Among the CBAVD patients, 8/50 (16%), 4/50 (8%), and 4/50 (8%) of subjects were heterozygote for DF508, G542X, and N1303K, respectively, accounting for 16% of all mutant alleles (Table 2 and Fig. 2).

In the screening of IVS8-Tn polymorphism, seven of the 50 CBAVD patients had the 5T/5T genotype, nine were heterozygous for the 5T/9T genotype, and 11 were heterozygous for the 5T/7T genotype. The frequency of 5T alleles was 34%. The number of patients with genotypes of 9T/9T, 9T/7T, and 7T/7T were 4, 7, and 12, respectively (Table 3). None of the CFTR gene mutations were detected in NOA patients, and the frequency of the 5T allele (5%) was similar to the control group. The final results of the IVS8-Tn genotype in NOA and CBAVD patients are presented in Table 3. The frequency of 5T, 7T, and 9T alleles in both groups of the patients and the control are shown in Table 4.

**RESULTS**

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**DISCUSSION**

About 97% of men with CF are infertile as a result of CBAVD and OA. In addition, defects in the CFTR gene may affect the process of spermatogenesis[19,20,23]. The frequency of mutations within the CFTR gene varies in different populations. DF508 is known as the most prevalent mutation in the world[28]. It has also been reported as the most common allele in Iranian CF patients. The other most common mutations are W1282X, G542X, R347H, and R117H[29,30]. Although numerous investigations have revealed the relationship of CFTR gene with infertility problem in populations all over the world, a limited number of such studies have been published in males with CBAVD and other types of infertility in Iran. Hence, the objective of the present study was to investigate the most prevalent mutations in CFTR gene in Iranian

**Table 1.** Intron 8 variation detectable by HpaI restriction digest

<table>
<thead>
<tr>
<th>Result of digested products</th>
<th>CF5T/CF9RR</th>
<th>CF7T/CF9RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restriction enzyme</td>
<td>HpaI</td>
<td>HpaI</td>
</tr>
<tr>
<td>IVS8-5T allele</td>
<td>237+22(+)</td>
<td>235+22(+)</td>
</tr>
<tr>
<td>IVS8-7T allele</td>
<td>261(-)</td>
<td>237+22(+)</td>
</tr>
<tr>
<td>IVS8-9T allele</td>
<td>263(-)</td>
<td>261(-)</td>
</tr>
<tr>
<td>IVS8 genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5T/5T</td>
<td>+/+</td>
<td>-</td>
</tr>
<tr>
<td>5T/7T</td>
<td>+/-</td>
<td>+/+</td>
</tr>
<tr>
<td>5T/9T</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>7T/7T</td>
<td>-/-</td>
<td>+/+</td>
</tr>
<tr>
<td>7T/9T</td>
<td>-/-</td>
<td>+/+</td>
</tr>
<tr>
<td>9T/9T</td>
<td>-/-</td>
<td>-/-</td>
</tr>
</tbody>
</table>

Each genotype has a unique HpaI digest pattern when both PCR products are considered.

**Table 2.** Frequency of mutations in CBAVD patients

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Genotype</th>
<th>CBAVD patients (n = 16)</th>
<th>Genotypic frequencies (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G542X</td>
<td>G542X/-</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>N1303K</td>
<td>N1303K/-</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>ΔF508</td>
<td>ΔF508/-</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>W1282X</td>
<td>/-</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>G551D</td>
<td>/-</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>
infertile men (CBAVD and NOA). Selection of the most common mutations (ΔF508, G551D, G542X, N1303K, and W1282X) was based on the high prevalence of these mutations reported in the CFTR gene worldwide and also based on the presence of these mutations in patients with CBAVD.[19,20]

In our CBAVD patients, ΔF508 with a frequency of 8% was detected as the most common mutation, and then N1303K and G542X (4% and 4%, respectively) were found, which was significantly greater than that of the control group (p < 0.001). The 5T allele showed a significantly higher rate compared to the control group (34% vs. 3%, p < 0.001). These results are consistent with several investigations that evaluated CFTR mutations in CBAVD, as well as mutations of ΔF508, 5T, and R117H as the prevalent variations.[6,16,31]

In 2007, Radpour et al.[32] published a paper in which different types of mutations were found in the CFTR gene among Iranian CBAVD patients. ΔF508 had a high prevalence in patients, and the frequency of IVS8-5T was 27.23%, which corresponds to the present investigation (34%). Approximately 35% of Iranian men with CBAVD are carriers for a single mutation or polymorphism in CFTR gene, and around 40% have two mutations (common CFTR mutations or IVS8-5T polymorphism).[32,33] The frequency of IVS8-5T in our patients was similar to that of Portuguese (27.4%)[34] and Taiwanese patients (29.2%)[35] but higher than Turkish (19.6%)[31].

In our study, the proportion of patients with NOA who had the 5T allele was the same as normal population (5 vs. 3; p = 0.721). No mutation was found in this group of patients and healthy normal samples. The findings of the present study have some similarities and differences to some previous studies. In the study performed by Heidari et al.[32] for screening two CFTR mutations (Δ507 and ΔF508) in Iranian men with NOA, the ΔF508 was found in three individuals; no statistically significant relationship was found between this mutation and NOA.[36] In a recent study, Jiang et al.[37] investigated the prevalence of ΔF508 and R117H mutations, IVS8 poly(T) and TG repeats, in Chinese NOA patients. Their result indicated no mutation in patients and fertile controls, suggesting that these two mutations have a low possibility of being associated with NOA condition. Besides, the T5 allele was identified as the most prevalent factor that increases the risk of having NOA in Chinese.

In the evaluation of CFTR gene mutations in males with various types of abnormal sperm parameters by Slezak et al.[38] who observed that ΔF508 and IVS8-T occurred in 5.4% of patients, which was similar to the general population. van der Ven et al.[21] have screened 127 males with poor sperm quality for 13 CFTR gene mutations. The frequency of mutations was significantly higher than the expected CF carrier frequency in the general population, and no mutations were detected in the control group, which was different from our work. In 2011, Safinejad et al.[39] evaluated the common CFTR mutations (ΔF508, G542X, R117H, W1282X, and N1303K) and the frequency of the 5T variant in men with non-CAVD OA. Their result revealed that 5/53(9.43%) and 4/53(7.55%) of patients were heterozygote for ΔF508 and G542X mutations, respectively, and 5T polymorphism was 14%, which was more frequent than the control subjects (3%; p < 0.05). In another research that was carried out on 1195 males with nonobstructive infertility and those who

<table>
<thead>
<tr>
<th>Alleles</th>
<th>5T</th>
<th>7T</th>
<th>9T</th>
<th>G542X</th>
<th>N1303K</th>
<th>ΔF508</th>
<th>G551D</th>
<th>W1282X</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOA (n = 50)</td>
<td>5/100</td>
<td>36/100</td>
<td>59/100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBAVD (n = 50)</td>
<td>34/100</td>
<td>42/100</td>
<td>24/100</td>
<td>4/100</td>
<td>4/100</td>
<td>6/100</td>
<td>0/100</td>
<td>0/100</td>
</tr>
<tr>
<td>Control (n = 100)</td>
<td>3/100</td>
<td>39/100</td>
<td>58/100</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Fig. 2. Detection of the most common CFTR mutations in CBAVD men by using multiplex ARMS-PCR. Lanes 1 and 2, fertile group (control); lane 3 and 4, heterozygote patients for ΔF508 mutation (ΔFn); Lanes 5 and 6, heterozygote patient for G542X mutation (GXm); Lane 7, 100 pb DNA ladder. NKn, ΔFn, and GXn represent the PCR products for normal alleles N1303K, ΔF508 and G542X, respectively.

unexplained infertility, no significant difference in CFTR gene was identified between the control group and patients. Sharma et al. have examined the frequency of CFTR mutations in Indian infertile patients with NOA and spermatogenic failure. They reported ΔF508 mutation in 3.6% of men with NOA. In this study, a significant correlation was detected between the most common mutations of the CFTR gene and IVS8-Tn polymorphism in CBAVD patients. However, the relationship between this gene and NOA remains under question. Thus, large-scale cohort studies as well as examination of entire gene or the high number of CFTR mutations may be necessary to substantiate the hypothesis of a putative link between a particular combination of CFTR mutations and polymorphisms and other types of male infertility.

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CONFLICT OF INTEREST. None declared.

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