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Common CFTR Polymorphisms in Infertile Men With Non CAVD Obstructive Azoospermia

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ABSTRACT

This study was designed to determine the frequency of cystic fibrosis transmembrane conductance regulator (CFTR) haplotypes poly-T and M470V polymorphisms in infertile men with non-congenital absence of vasdeferens.
(Non-CAVD) obstructive azoospermia as patients who had been referred to the Royan-2 infertility center, and to compare their distribution with that in the fertile men as control individuals .Genomic DNA was extracted from 53 patients and 50 control individuals blood leukocytes. Intron8/exon9 and exon 10 PCR-amplified products were digested by HpaI and HphI restriction enzymes respectively. T7 was the most common haplotype , and T7/T7 was a dominant genotype in the patients and control individuals . The frequency of the 5T variant was 15 of 106 alleles(14%) which was more frequent than control individuals(3%)($p<0.05$). The most patients with T7 allele were associated with the M470 variant but in control individuals T7 allele were associated with the V470 variant and difference between these haplotype frequencies in the patients and control individuals were significant. The frequency of poly-T and the M470V haplotype polymorphisms identified in these patients differs significantly from the frequency found in the control individuals.
KEYWORDS: Cystic fibrosis transmembrane conductance regulator gene, haplotype frequencies, Poly-T, M470V, Non- CAVD obstructive azoospermia

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.

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 [4]. The CFTR gene on chromosome 7q31 spans approximately 250kb of DNA and encodes 27 exons [5].

The gene encodes a cAMP- and ATP-dependent CFTR chloride channel that is present in the membrane of the epithelial cells that line most exocrine glands[6] .

Phosphorylation of the regulatory domain by protein kinase A, followed by binding and hydrolysis of ATP at both nucleotide-binding domains, regulates the transport of chloride ions through the channel[7] .

Absence, reduced levels, or malfunction of the CFTR protein results in cystic fibrosis (CF), and CF-like diseases such as congenital bilateral absence of the vas deferens (CBAVD) [8, 9] , bronchiectasis [10] and chronic pancreatitis[11] .

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Poly T and M470V polymorphisms play a role in the development of CF-like diseases. The poly-T tract located at the junction of intron 8(IVS-8) and exon 9 influence transcription and thereby reduce the amount of normal CFTR protein.The number of T residues present,five,seven and nine affects the splicing efficiency of exon 9.If the T5 allele is present, a proportion of CFTR transcripts will lack exon 9, which produces a non-functional protein and variable CF symptoms [12].

On the other hand, the M470V polymorphism on exon 10 affects the intrinsic chloride activity, and thereby affects the function of the CFTR protein[13,14].

There are just a few data on CFTR in Asia, especially in the Iran, and on common CFTR polymorphisms in infertile men with non- CAVD obstructive azoospermia no data have been reported, especially in the Iran.

The aim of this study was to evaluate the frequency of poly-T and the M470V haplotype polymorphisms in the 53 Infertile men with non-CAVD obstructive azoospermia and 50 control individuals .

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 -2 Infertility Center in Qom, Iran and from 50 normal men(men with normal fertility and sperm parameters). 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 insufficiency and intestinal obstruction have been reported in clinical file of our patients.

Genomic DNA was extracted from the whole blood by a non-organic method involving lysis, proteinase K digestion and salting out of DNA with isopropanol precipitation [15 ,16].

The CF intron 8/exon9 (product size, 259 to 261bp) primers were as follows: common downstream primer, CF9RR:GACATGGACACCAAATTAAG;upstream primer, CF5T:GTGTGTGTGTGTGTGT-GTTG*TT (*denotes mismatch); and upstream primer, CF7T:GTGTGTGTGTGTGTGTTTTG*TT [17].

Intron8/exon9 and exon 10 PCR-amplified products by digested by HpaI and HphI restriction enzymes respectively and sequencing for evaluate of haplotype frequencies in compound heterozygote individual.

PCR program for amplifications of exon 10 began with a 5 min incubation at 94°C,and Proceeded with 28 cycles, each containing 15s of denaturation at 94°C,30s of annealing at 60 °C and 30 s of extension at 72°C;with a 10 min incubation at 72°C. PCR program for amplifications of Intron8/exon9 began with a 2.5 min incubation at 94°C, and proceeded with 34cycles, each with 1min of denaturation at 94°C, 1min of annealing at 58°C and 1min of extension at 72°C; with a 10 min incubation at 72°C.PCR conditions for amplification of above DNA samples stood as described earlier [18],260 to 264 bp (intron8) or 510bp(exon10)was subsequently digested with 1U Hpa I (intron8)or HphI (exon 10) for 3 hours or overnight at 37°C.After digestion,products were run on an 8% acrylamidegel with 0.5×Tris-borate-ethylene- Diamine tetra acetic acid(TBE)at 200V for 2hours 45 minutes.

Analysis of data:

Differences in polyT alleles and M470V frequencies between patients group and control group were compared by the chi square statistic test (SPSS software version 16.0) and P-values < 0.05 were considered to indicate statistical significance.

RESULTS

Poly-T:

T7 was the most common haplotype, and T7/T7 was a dominant genotype in the patients and control individuals. In the patients fifteen alleles of T5 with a frequency of 14%were found , T9 alleles were very rare at just 9.5%.7T/9T heterozygotes in the patients was slightly less frequent than control individuals and5T/7T heterozygotes in the patients was slightly more frequent than control individuals. None of the control individuals were found to carry a 5T /5T ,9T/9T and 5T/9T genotypes.

The allele distribution was 14 and 3% for 5T ,76.5and 87% for 7T and 9.5and 10% for 9T for the non-CAVD men and normal men, respectively (Table 1,2) .

Table – 1:Poly T genotype frequencies in the patients and control individuals

5T/5T (%)	5T/7T (%)	5T/9T (%)	7T/7T (%)	7T/9T (%)	9T/9T (%)	Genotypes / Groups
1(2)	11(21)	2(4)	33(62)	4(7)	2(4)	Patients(n=53)
0	3(6)	0	37(74)	10(20)	0	Control individuals (n=50)

Table- 2: Poly T allele frequencies in the patients and control individuals

5T (%)	7T (%)	9T (%)	Frequencies / Groups
15(14)	81(76.5)	10(9.5)	Patients(2n=106)
3(3)	87(87)	10(10)	Control (2n=100)

M470V:

The V allele was slightly more frequent than the M allele and dominant genotype was M/V, followed by V/V and M/M in the patients and control individuals .In our study, the percentage of patients who had MM and VV genotypes were significantly higher than the normal men ($P < 0.05$) (Table 3,4) .

Table – 3: M470V polymorphism genotype frequencies in the patients and control individuals

MM (%)	MV (%)	VV (%)	Genotypes / Groups
10(19)	30(57)	13(24)	Patients(n=53)
11(22)	20(40)	19(38)	Control(n=50)

Table – 4 :M470and V470 allele frequencies in the patients and control individuals

M (%)	V (%)	Frequencies / Groups
47	53	Patients(2n=106)
42	58	Control (2n=100)

Poly-T and M470V:

Analysis of polyT with M470V showed that 7T/M470 was the most common haplotype (48.2%)in patients, 7T/V470 was the most common haplotype (54%)in control individuals .

In our study,differences between the percentage of 7T/M470 and 7T/V470 haplotypes in patients and control individuals were significant ($P < 0.05$) .

The percentage of 5T/M470 and 5T/V470 haplotypes in the patients were significantly higher than the control individuals ($P < 0.05$).Also the percentage of 9T/M470 and 9T/V470 haplotypes in the patients and control individuals were not significant ($P > 0.05$) (table-5).

Table-5: haplotype frequencies of Poly-T and M470V Polymorphism in the patients and control individuals

haplotype	**Patients(n=53)**		**Control individuals (n=50)**	
	n=106	frequency (%)	n=100	frequency (%)
7T/M	51	48.2	34	34
7T/V	31	29.2	54	54
9T/M	5	4.7	7	7
9T/V	5	4.7	2	2
5T/M	7	6.6	2	2
5T/V	7	6.6	1	1

DISCUSSION

In order to study the possible involvement of CFTR dysfunction in non-CAVD obstructive azoospermia men from Iran, a country with presumed low frequency of CF, we have performed the first study of comparative analysis of common CFTR polymorphisms poly-T and M470V in the 53 infertile men with non-CAVD obstructive azoospermia and 50 control individual.

In all studies performed so far, a high frequency of CFTR mutations or the 5T variant has been found in the patients with CAVD[9,19,20,21] but reported studies in patients with Non CAVD is very few especially in Iran.

There are reports that CFTR is also involved in non-CAVD obstructive azoospermia (other forms of obstructive azoospermia than CAVD) [20,22,23,24,25].

Studies have shown that the frequency of the 5T variant was significantly higher in men with CBAVD (46%), congenital unilateral absence of the vas deferens(CUAVD) (25%), or epididymal obstruction (29%) than the general population (5%)[9,19,20,21].

In our patients the frequency of the 5T variant , a variant frequently found in Caucasian CBAVD men [9,19,20,21, 26,27], was 15 of 106 alleles (14%) which was more frequent than control individuals (3%) ($p<0.05$) (Table 2).

Our study is consistent with some studies that have been previously reported[20].Indeed, it appears that azoospemiic men with idiopathic epididymal obstruction have a significantly higher frequency of the 5T variant (29%) compared to the general population (5%)[20].

The M470V polymorphism located in exon10 has also been reported as a modifier element of the penetrance of the 5 T allele,with the V470 variant being involved in lower normal CFTR protein levels[13,28,29]. In our study, the V470 variant was found in 43 of 53 patients(81%),with an allelic frequency of 53% (56/106) (table-4).The most patients with non CAVD with 7T allele were associated with the M470 variant but in the control individuals T7 allele were associated with the V470 variant and differ between these haplotypes frequencies in the patients and control individuals were significant(table-5).

Despite extensive investigation of the CFTR gene, the frequency of some of polyT / M470V haplotypes identified in this cohort of patients differ significantly from the frequency found in the control individuals .

Thus, although a particular allele may not by itself have deleterious consequences, the combination of specific alleles at several polymorphic loci might result in less functional or even insufficient CFTR protein. It was postulated [13] that such polyvariant mutant genes might be involved in the partial penetrance of CFTR gene mutations (such as the 5T allele), might be responsible for variations in the phenotype of CFTR mutations, and could explain why apparently normal CFTR genes cause disease. If confirmed by further analysis in other populations, these findings will have consequences with regard to the genetic counselling provided to infertile patients treated by in-vitro fertilization.

CONCLUSIONS

Analysis of haplotype T7-M470 in a larger cohort will be necessary to substantiate the hypothesis of a putative link between a particular combination of CFTR polymorphisms and male infertility. Further study of the relationship between polymorphisms of poly-T and M470V haplotypes in Non CAVD infertile men should be undertaken.

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