original article

Cystic fibrosis gene mutations and polymorphisms in Saudi men with infertility

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Citation: AlMaghamsi T, Iqbal N, Al-Esai NA, Mohammed M, Eddin KZ, Ghurab F, et al. Cystic fibrosis gene mutations and polymorphism in Saudi men with infertility. Ann Saudi Med 2020; 40(4): 321-329 DOI: 10.5144/0256-4947.2020.321

Received: March 30, 2020

Accepted: June 13, 2020

Published: August 6, 2020

Funding: None.

BACKGROUND: Some mutations of the cystic fibrosis transmembrane regulator (*CFTR*) gene may impair spermatogenesis or cause a congenital absence of the vas deferens that manifests as isolated male infertility.

OBJECTIVE: Assess the frequency and analyze the spectrum of *CFTR* gene variations in Saudi men with primary infertility.

DESIGN: Prospective, cross-sectional.

SETTING: Tertiary care specialist hospital in Jeddah.

PATIENTS AND METHODS: Genomic DNA was extracted from peripheral blood samples of Saudi men who presented with primary infertility to the outpatient andrology clinic with either azoospermia or oligoasthenoteratozoospermia. Polymerase chain reaction and direct sequencing were used to identify all variants of the *CFTR* gene.

MAIN OUTCOME MEASURES: Proportion of the patients with a mutant *CFTR* gene and the spectrum of *CFTR* gene variations.

SAMPLE SIZE: 50 infertile Saudi men.

RESULTS: This study identified 10 CFTR gene variants in 7 (14%) subjects (100 chromosomes). The detected variants and polymorphisms were: c.1408G>A, c.4389G>A, c.2562T>G, c.869+11C>T, c.2909-92G>A, c.3469-65C>A, c.1210-6delT, c.1210-6T>A, c.2988+1G>A, and c.1210-13GT>TG.

CONCLUSION: We demonstrated that 14% of the study subjects had one or more *CFTR* mutations and these were compounded in most of the affected patients. The spectrum of *CFTR* gene mutations in these subjects was similar to the mutations reported in other studies throughout the world.

LIMITATIONS: Small sample size and the lack of a control group.

CONFLICTS OF INTEREST: None.

Cystic fibrosis (CF) is a progressive disease affecting the exocrine secretions of many organs. The prevalence and the carrier frequency of CF vary according to the ethnic background and degree of consanguinity.[1] CF affects approximately 1 in 2500 individuals with an average carrier frequency of 1:25 in populations of European and Middle Eastern descent.[2] The exact incidence of CF in Saudi Arabia is unknown. Based on classical clinical features and elevated sweat chloride concentrations (>60 mmol/L), Nazer et al reported that CF occurs 1 in 4243 of Saudi live births.[3]

CF is an autosomal recessive disorder caused by mutations of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene (OMIM 602421).[4] This 250-kb gene was mapped to the long arm of chromosome 7 at position 31.2.[5] In the past, it was thought that this gene contained 24 exons.[6] Subsequently, it became apparent that it contains 27 exons and 26 introns as exons, 6b, 14b, and 17b were missed in the original publication. The *CFTR* gene encodes a 1480-amino acid protein called *CFTR*.[7] This protein has regulatory functions as a channel that regulates the flow of salt and fluids in and out of the cells that produce mucus, sweat, saliva, tears, digestive enzymes, and semen.[8] Due to its large size, the *CFTR* gene is highly susceptible to frequent mutations.[9] Some mutations/variants in the *CFTR* gene may result in the absence or abnormal *CFTR*. Thus, the movement of salts into and out of cells is hindered, leading to the formation of thick and sticky mucus. This glue-like mucus builds up and causes problems in many exocrine organs, especially the lungs, pancreas, and reproductive tract.

There are 2074 variants listed in the *CFTR* mutation database with wide variability in phenotype expression.[10] Those affected may have classical CF or CF-like diseases such as the congenital absence of the vas deferens, bronchiectasis, or chronic pancreatitis.[11-13] Male infertility is one of the hallmarks of *CFTR* mutations. Many studies involving DNA mutation analyses of the *CFTR* gene have demonstrated that infertile men with obstructive azoospermia from congenital bilateral absence of vas deferens (CBAVD), congenital unilateral absence of vas deferens, epididymal obstruction, or severe oligoasthenoteratospermia (OAT) have a considerably higher incidence of mutations in the *CFTR* gene compared with the general population.[14-20]

This important observation has not been evaluated in the Saudi population where it is believed to be rare. In addition, identification of *CFTR* gene variants in infertile Saudi men may play a role in genetic counseling of these index cases or studying the genotype/phenotype correlations. Knowledge of the actual mutations could also be important for targeting treatments of these cases in the future. Therefore, this study aimed to examine the frequency and analyze the spectrum of *CFTR* gene variants in infertile Saudi men with oligoasthenoteratozoospermia (OAT) or azoospermia (AZO) who were otherwise healthy.

PATIENTS AND METHODS

This was a prospective cross-sectional study conducted at KFSHRC-Jeddah in collaboration with the Saudi Molecular Diagnostic Laboratory (Saudi MDL) at the King Faisal Specialist Hospital and Research Center KFSHRC-Riyadh, Saudi Arabia. KFSHRC-Jeddah is the largest tertiary care referral facility in the western region of Saudi Arabia. The Saudi MDL is fully accredited by the College of American Pathologists, United States.

The study population consisted of 50 adult Saudi men with OAT or AZO who presented with primary infertility to the outpatient andrology clinic at King Faisal Specialist Hospital and Research Center-Jeddah (KFSHRC-J), Saudi Arabia, between January 2017 and January 2019. Only patients who were apparently unrelated were included in this study. The diagnosis of primary infertility was based on clinical evaluation and semen analysis. Additional workups, such as semen cultures, scrotal ultrasonography, and testicular biopsies, were conducted when appropriate. Patients with bilateral orchiectomy, chromosomal defects, or a history of exogenous spermatotoxic insults (mumps orchitis, injured reproductive organs, scrotal tumors, or exposure to chemicals or radiation) were excluded.

After appropriate genetic counseling, each eligible subject was asked to participate in this study. When accepted, informed consent for molecular genetic analyses was obtained from each subject. Demographic, clinical, and socioeconomic data, and family and surgical histories were collected from each subject using a specific data-collection form. A blood sample for *CFTR* gene mutation analysis was obtained from each patient and, if not previously obtained, another blood sample for serum prolactin, follicle-stimulating hormone, luteinizing hormone, and testosterone levels were collected and analyzed. One or more semen samples were also obtained from each subject for semen analysis. The study project and written informed consent form were approved by the institutional review board at KFSHRC-Jeddah (IRB approval number 2016-25/RC-J/6/38).

Intervention and mutation analysis

Semen samples were collected via masturbation after four days of sexual abstinence and the semen analysis was conducted in the andrology laboratory at our

institution. The specimens were classified according to the World Health Organization Laboratory Manual for the Examination and Processing of Human Semen, 5th Edition (WHO 2010).[21] Severe oligozoospermia was considered when the sperm concentration was less than 5 million sperm/mL and azoospermia was considered when sperm were absent in at least one ejaculate semen sample. Hormone assays were conducted via immunoassay for the in vitro quantitative determination in human serum and plasma. The results of the semen analysis, hormone levels, and a genetic test for each subject were collected from the patient's electronic chart with the corresponding data form.

A peripheral venous (3-4 mL) blood sample from each patient was collected in EDTA tubes. Each specimen container was labeled with the medical record number of the subject before being shipped to the Saudi MDL. Testing for mutations in the *CFTR* gene was conducted via polymerase chain reaction (PCR) and direct sequencing. Genomic DNA from the leukocytes of each individual was used to amplify the 27 coding exons and their flanking introns using primers designed by Primer 3 software (*http://frodo.wi.mit.edu/*) and synthesized by Metabion International AG (Munich, Germany). PCR was conducted on a final volume of 25 µL containing approximately 20 ng of genomic DNA and a Qiagen (Manchester, UK) master mix kit (including 1×PCR buffer, 100 mmol/L dNTP, and 1 U per reaction HotStar Taq polymerase) and 0.5 mmol /L primers. The PCR products were treated with the Agencourt AMPure PCR purification system (Agencourt Bioscience Corporation, Beverly, MA, USA). The PCR products were sequenced using the BigDye Terminator Cycle Sequencing kit (PE Applied Biosystems, Beverly, MA, USA) according to the manufacturer's instructions. The sequences were analyzed using Mutation Surveyor software version 3.24 (SoftGenetics LLC, State College, PA, USA). Using this sequencing technique, the coding and flanking intronic regions of the *CFTR* gene had high sensitivity and specificity values, and up to 98.7% of the *CFTR* mutations were covered. Excluding sampling errors and sample contamination, the sequence variant detected in both strands of an amplicon was over 99.9% specific.[22] The nucleotides were numbered according to the Nomenclature Working Group recommendations, with the "A" of the initiation codon (ATG) counted as nucleotide 1.23 When detected, the deleterious sequence variants, small insertions, or deletions in the coding regions or splice junctions in the *CFTR* gene were reported. The sequencing results were then searched in the dbSNP database (*http://www.ncbi.nlm.nih.gov/snp/*) to obtain the corresponding reference SNP and HGVS nomenclature.

We calculated the proportion of the patients with a mutant *CFTR* gene. The baseline characteristics, hormonal levels, and semen parameters were compared between the subjects with normal and abnormal *CFTR* genes. Fisher's exact test was used to assess the associations between categorical variables, while the *t* test, non-parametric tests including Kruskal-Wallis, and sign tests were used accordingly to assess the associations between continuous variables. The statistical analyses were conducted using SAS for Windows software (Statistical Analysis System, version 9.4; SAS Institute Inc., Cary, NC, USA). All of the *P* values were based on two-sided comparisons and *P* values <.05 were considered statistically significant.

RESULTS

Clinical characteristics

This study encompassed 50 Saudi male subjects diagnosed with primary infertility (**Table 1**). Their ages ranged between 26 and 46 years (mean: 35.4 [95% CI: 34.0 and 36.7]) which covers the usual ages of men being investigated for infertility. All of the patients were native Saudis, apparently unrelated, and the parents of 54% of the subjects were in consanguineous marriages. Azoospermia was diagnosed in 54% (27/50) and OAT in 46% (23/50) of the patients. Among the 50 patients, five had a history of chronic cough (3 diagnosed as bronchial asthma, one as idiopathic bronchiectasis, and one as an unknown cause) but none had a recurrent lung infection or chronic diarrhea. None of the subjects had previously been diagnosed with CF. Seven patients (14%) had undergone varicocelectomy, one had vasoepididymostomy, three underwent unilateral orchidopexy during adulthood and three (6%) had undergone inguinal hernia repairs. No significant difference was found between those who carried *CFTR* mutations and those who did not with respect to age, weight, and other clinical characteristics.

The semen volumes for all of the subjects were between 0.2 and 6.4 mL with a median of 2.0 mL. When the semen parameters of the subjects with abnormal genes were compared with those with normal genes, no statistically significant difference was found. The median level (9.0 nmol/L) of the serum testosterone hormone in the subjects with abnormal genes was significantly lower (*P*=.009) than in those with no mutations (13.2 nmol/L), while there were no differences in other hormones (**Table 2**).

CFTR *mutation patterns and frequencies*

In 100 screened chromosomes, 10 different *CFTR*

Table 1. Clinical characteristics of the study population.

	All subjects (n=50)	Subjects with abnormal *CFTR* gene variants (n=7)	Subjects with normal *CFTR* gene variants (n=43)	*P* value
Age (y)	35.4 (5.1), 26-46	37.9 (4.1), 35-46	35.0 (5.1), 26-45	.17
Weight (kg)	88.0 (25.6)	81.6 (9.4)	89.1 (27.2)	.18
CF features[a]	5a (10%)	1	4	.54
Parental consanguinity	27 (54%)	3	24	.68
Family history of primary infertility[b]	29 (58%)	3	26	.43
Chronic conditions	17 (34%)	3	14	.67
Diabetes mellitus	5 (10%)	2	3	-
Chronic asthma	3 (6%)	0	3	-
Bronchiectasis	1 (2%)	1	0	-
Hypoplastic testis	1 (2%)	0	1	-
Other unrelated condition	7 (14%)	0	7	-
Prior related surgeries	14 (28%)	1	13	.65
Varicocelectomy	7 (14%)	1	6	-
Inguinal hernia repairs	3 (6%)	0	3	-
Scrotal or testicular surgeries	4 (8%)	0	4	-

Data are number (%) or mean (standard deviation). [a]Five patients have a chronic cough, 3 diagnosed with BA and 1 with bronchiectasis, one no unclear cause; [b]Positive family history of primary infertility either in brothers, sisters, grandfathers, grandmothers, uncles, or aunts. CFTR: Cystic fibrosis transmembrane regulator.

gene mutations and polymorphisms were detected in 7 patients (14.0% [95% CI: -4.4 to 23.6%]). Six of the 7 patients (12% of the total cases [95% CI: 3.0%-21.0%]) had two or more heterozygous variants while one had a single homozygous mutation. The mutations were observed in 4 patients with azoospermia (14 % [95% CI: 0.91% to 27.09%]) and in 3 (13% [95% CI: -0.74% to 26.74%]) with severe OAT. Interestingly, one azoospermic patient had 4 variants, 3 heterozygous alleles, and one (c.2988+1G>A) homozygous allele. The variants and polymorphisms detected were c.1408G>A, c.4389G>A, c.2562T>G, c.869+11C>T, c.2909-92G>A, c.3469-65C>A, c.1210-6delT, c.1210-6T>A, c.2988+1G>A, and one novel variant (c.1210-13GT>TG) (**Table 3**).

All of the identified variants were single-nucleotide polymorphisms, three were coding sequences (one missense and two synonymous), and the remainder were intronic (two of them splicing). Seven variants were previously reported as significant, while two (c.3469-65C>A and c.1210-6T>A) were not. One variant was novel (c.1210-13GT>TG) and was detected as a heterozygous form in combination with three other variants in one patient. This azoospermic patient had undergone a varicocelectomy and had a family history of infertility, a higher level of serum gonadotropin hormones, lower testosterone, and his testicular sperm extraction showed no significant pathological changes.

The most commonly detected variants were c.1408G>A and c.4389G>A. Each of these variants was homozygous in one patient and heterozygous in two patients with an allelic frequency of 4%. Variants c.2562T>G and c.869+11C>T were next and each was heterozygous in two patients.

DISCUSSION

Several studies from different countries have confirmed that mutations in the *CFTR* gene are directly involved in the pathogenesis of male infertility, especially in some patients with obstructive azoospermia, or testicular failure.[11,24-27] However, the spectrum and frequency distribution of *CFTR* gene mutations in the Saudi population is unknown. Therefore, this study was conducted to identify the spectrum and frequency of *CFTR* gene mutations in infertile Saudi males. We found that the frequency of *CFTR* mutations in 50 infertile Saudi men

Table 2. Semen analysis, sperm parameters, and hormones level in Saudi Infertile men.

Variable	All Subjects (n=50)	Subjects with Abnormal *CFTR* variants (n=7)	Subjects with Normal *CFTR* variants (n=43)	*P* value
Semen analysis				
Ejaculated volume (mL)	2.0 (2.4)	2.8 (2.8)	2.0 (2.4)	.37
Number (%) with volume <1.5 mL	19 (38)	2 (28)	17 (40)	.69
Semen pH	8.0 (1.0)	8.0 (0)	8.0 (1.0)	.57
Number with with pH 7.0 – 8.0	37 (74)	6 (86)	31 (72)	.65
Sperm concentration (million/mL)	0 (1.0)	0 (2.0)	0 (1.0)	.96
Total sperm count (million/ ejaculate)	0 (1.0)	0 (8.0)	0 (1.0)	.94
Sperm motility (%)	0 (16.0)	0 (14.0)	0 (16.0)	.63
Number (%) with sperm motility <40%	46 (92)	7 (100)	39 (90)	1.00
Hormones levels				
FSH (IU/L)	6.85 (7.5)	16.3 (16.0)	6.5 (5.6)	.06
Number (%) with abnormal level[a]	12 (24)	6 (86)	6 (14)	.00
LH (IU/L)	6.8 (3.9)	10.9 (9.0)	6.3 (2.8)	.07
Number (%) with abnormal level[b]	17 (34)	6 (86)	11 (26)	.004
Prolactin (ug/L)	10.5 (6.8)	10.6 (7.1)	10.3 (6.8)	.64
Number (%) with abnormal level[c]	10 (20)	1 (14)	9 (20)	1.00
Testosterone (nmol/L)	12.2 (6.7)	9.0 (5.9)	13.2 (8.0)	.009
Number (%) with abnormal level[d]	18 (36)	5 (71)	13 (30)	.08

Data are median (interquartile range) unless indicated otherwise. [a]Above or lower than the normal range (1.5–12.4 IU/l); [b]Above or lower than the normal range (1.7–8.6 IU/l); [c]Above or lower than the normal range (2.1–17.7 ug/L); [d]Above or lower than the normal range (9.9–27.8 nmol/l). FSH: follicular stimulating hormone, LH: Luteinizing hormone.

was 14.0%. Among the affected patients, 6 out of 7 (85%) were carriers for two or more variants.

The higher *CFTR* mutation frequency in patients with AZO and severe OAT was supported by similar findings in several studies. In concordance with our findings, Van der Ven et al[15] found that 14 of 80 (17.5%) healthy men who had infertility due to reduced sperm quality and 3 of 21 (14.3%) men with azoospermia had at least one CF mutation. Our observed frequency of *CFTR* mutations was lower than that reported by many investigators in Europe, Iran, and Canada.[17,28-31] For example, in a large case-control study, Gallati et al found that *CFTR* mutations were detected in 68% of CAVD, 31% of the azoospermic, and 22% of the oligospermia men.[28] Similarly, Dörk et al investigated a cohort of 106 German patients with congenital absence of vas deferens CAVD and reported that 75% of the patients carried one or more disease-associated *CFTR* variants.[29] Another large study by Mercier et al analyzed 67 congenital bilateral absence of vas deferens patients from different European countries and showed that 42% of the subjects were carriers of one *CFTR* allele and 24% were compound heterozygous.[30] Radpour et al investigated 112 Iranian men with CBAVD and showed that 41% had compound mutations in the *CFTR* gene.[31] Jarvi et al studied 62 Canadian men with obstructive azoospermia of unknown etiology and showed that at least 47% had either known *CFTR* mutations or the 5T allele.[17] It should be noted that the higher frequency of *CFTR* mutations in all of these studies may have been because the majority of the patients had CAVD. In contrast, several articles reported lower rates varying between 5% and 11.6%. For instance, in a large cohort study, Shulz et al analyzed 597 German males with

Table 3. Details of detected ten *CFTR* variants.

Genomic location (GRCh37.p13 Chr 7)	Reference SNP (rs ID)	HGVS Name	Legacy name	Type of variant	#Exon/intron	Amino acid changes	Clinical significance in ClinVar	Number of patients with variant	Allele frequency in gnomAD v2.1
117199533G>A	rs213950	c.1408G>A	M470V	Coding Sequence, Missense	Exon 11	p.Val470Met	Reported	3	0.4865
117307108G>A	rs1800136	c.4389G>A	4521G/A	Coding Sequence, Synonymous	Exon 27	p.Gln1463=	Reported	3	0.2206
117235055T>G	rs1042077	c.2562T>G	T854T	Coding Sequence, Synonymous	Exon 15	p.Thr854Thr	Reported	2	0.3901
117176738C>T	rs1800503	c.869+11C>T	1001+11C/T	Intronic	Intron 7	NA	Reported	2	0.0693
117246636G>A	rs35050470	c.2909-92G>A	3041-92G/A	Intronic	intron 17	NA	Reported	1	0.1755
117267511C>A	rs213989	c.3469-65C>A	3601-65C/A	Intronic	Intron 21	NA	Not Reported	1	0.1972
117246808G>A	rs75096551	c.2988+1G>A	3120+1G>A	Splice Donor	Intron 18	NA	Reported	1	0.0001169
117188689delT	rs1805177	c.1210-6delT	-	Indel Intronic	Intron 9	NA	Not Reported	1	-
117188689T>A	rs763339686	c.1210-6T>A	-	Intronic	Intron 9	NA	Reported	1	0.0000406
117188682GT>TG	-	c.1210-13GT>TG	-	Splicing Intronic	Intron 9	NA	Novel variant	1	-

GRCh37; Genome Reference Consortium Human Build 37, Gln: Glutamine, GnomAD; The Genome Aggregation Database, HGVS; Human Genome Variation Society, Met; Methionine, NA; non available, Thr; Threonine, Val; Valine.

abnormal semen parameters for nine different *CFTR* mutations and found that a heterozygous variant was observed in 5.7% of the cohort.[32] Sharma et al reported that 11.6% of non-obstructive azoospermic men and 7.3% of those with spermatogenic failure had abnormal variants.[33] Similarly, in 100 infertile Chinese males, the T5 allele was present in 5%.[34] It should be noted that the genetic tests for *CFTR* mutations in the majority of these studies that had lower rates screened only for the most frequent CF causing mutations and not the milder mutations, and therefore may have resulted in lower *CFTR* mutation detection rates. Although many articles confirmed this association, three studies showed no statistical difference between *CFTR* carrier frequency and male infertility.[35-37]

No control group was available for this study, which is its main limitation, and the carrier frequency of the *CFTR* gene in the Saudi general population is unknown, making us unable to compare our findings with the corresponding results in our general population. Nazer et al reported that the incidence of classical CF (based on clinical manifestations and the sweat chloride test) in the Saudi population was 1:4,2.3 Based on the Hardy-Weinberg equation, a *CFTR* heterozygosity of 3% was estimated, which is similar to that reported worldwide. This indicates that the frequency of mutations in the *CFTR gene* in our sample was approximately four-fold higher than in the general population.

The congenital form of CF is caused either by homozygous variants, compound heterozygosity for one typical CF mutation with atypical mutations, or compound heterozygosity of atypical mutations.[38,39] Approximately 70% of these mutations in European descendants are F508del.[4] Although it is the most common mutation in 14 different Arabic countries, this mutation is not common in Saudi Arabia.[40] In addition to F508del, the T5 allele, IVS9 (intron 9) poly-T, TG repeats, R117H, and M470V polymorphisms are also common among patients with male infertility.[15,18,34] Our study identified 10 variants and polymorphisms that did not include F508del mutations. Our most frequent variants were c.1408G>A and c.4389G>A (an allelic frequency of 4% for each). Allele frequencies of all identified variants are presented in **Table 3**.

It was reported that the best characterized CBAVD variants are those that belong to poly-T, TG repeats (Tn and TGn tract), and M470V polymorphisms. Various numbers of dinucleotide TG repeats (TG9 to TG13) followed by T repeats (T5 to T9) were found in intron 9. Interestingly, the presence or absence of exon 10 in *CFTR* mRNA depends on the size of these sequences. For instance, the occurrence of low numbers of T

residues adjacent to high numbers of TG repeats (for example, T5-TG13) within these polymorphic tracts was substantially associated with alternative splicing and thus lacking exon 10 and generating a non-functional *CFTR* protein that manifests as obstructive azoospermia.[41-43] One azoospermic patient in our group had complex alleles containing one homozygous (c.2988+1G>A) splice donor mutation combined with 3 variants (c.1210-6delT, c.1210-6T>A, and c.1210-13GT>TG) of the poly-T-TG tract. This combination most likely correlated with his azoospermia.

The c.1408G>A (p. Val470Met) is a missense mutation in which guanine is replaced with adenine, resulting in a codon that codes for methionine instead of valine amino acid. By in vitro studies, it was shown that the *CFTR* gene carrying the V allele yielded a lower functional *CFTR* protein rate than those carrying the M allele.[42] The clinical role of this variant was assessed in several studies. Kosova et al studied the fertility effects of this variant in healthy European men and found that the homozygosity of this allele was associated with lower birth rates.[44] Further when the haplotype TG-T/M470V was considered, statistical analysis showed that the TG12-5T-V470 genotype was significantly associated with CBAVD (52.63%) compared to normal controls.[45]

We detected two synonymous coding variants (c.4389G>A and c.2562T>G). These variants have no effects on *CFTR* protein synthesis and were reported as benign.46-48 However, de Cid et al reported that patients with c.4389G>A haplotypes may present with a major clinical expression of CF-related disorders.[49]

The c.2988+1G>A (legacy name: 3120+1G>A) is a splice donor variant reported previously in those of African descent and in the Middle East.[50,51] Patients carrying these mutations often have variable disease expression, ranging from minimal lung disease to relatively severe disease in all of the involved organs.[52]

This study had limitations that merit mention, such as the small sample size, lack of a control group, and single center. Our sequencing technique could not detect large deletions-duplications variants, and we did not check whether the mutations are in cis or in trans configuration which is another limitation for this study. However, this is the first study to address the frequency and spectrum of *CFTR* gene mutations in infertile Saudi men, which provides preliminary data to justify conducting large cohorts or case-control trials in the future. Based on these findings, we suggest that genetic counseling and screening for *CFTR* mutations may be advised for these patients.

In summary, this study determined the frequency of *CFTR* gene variants in infertile Saudi men. We demonstrated that 14% of the study subjects had one or more *CFTR* mutations and were compound in the majority of the affected patients. The spectrum of the detected *CFTR* gene mutations in our subjects was similar to the levels reported worldwide. This frequency seems higher than in the general population and thus there may be an association between CFTR gene mutations and male infertility. Further analysis of the *CFTR* gene in multicentric large studies are required to assess this association in the Saudi population.

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