ANDROLOGY

ISSN: 2047-2919

REVIEW ARTICLE

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Keywords:
assisted reproductive techniques, *CFTR*, congenital bilateral absence of the vas deferens, cystic fibrosis, genetic counseling, infertility

Received: 10-Aug-2016
Revised: 2-Sep-2017
Accepted: 7-Nov-2017

doi: 10.1111/andr.12450

Congenital bilateral absence of the vas deferens as an atypical form of cystic fibrosis: reproductive implications and genetic counseling

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SUMMARY

Congenital bilateral absence of the vas deferens (CBAVD) is found in 1% to 2% of males with infertility and is present in 6% of obstructive azoospermia cases. Nearly 95% of men with cystic fibrosis (CF, an autosomal recessive disorder) have CBAVD. There are genetic links between CBAVD and CF. Some mutations in the gene encoding cystic fibrosis transmembrane conductance regulator (*CFTR*) can lead to CBAVD as a monosymptomatic form of CF. With the use of assisted reproductive techniques (ART), especially testicular or epididymal sperm aspiration, intracytoplasmic sperm injection, and in vitro fertilization, it is possible that men with CBAVD can produce offspring. Therefore, genetic counseling should be offered to couples undergoing ART to discuss the probability of having offspring that carry *CFTR* gene mutations. The aim of this review was to present the main cause of CBAVD, to call attention to its implications for assisted reproduction, and to show the importance of genetic counseling for couples where men have CBAVD, as they can have offspring with a lethal disease.

INTRODUCTION

About one in every six to ten couples has fertility problems. Subfertility originates from males in 20–25% of the cases, from females in 30–40% of the cases, and from both in 30% of the cases. The causes of subfertility remain unknown in 15% of the cases (World Health Organization, 1997). Among the 20–25% of males with subfertility, CBAVD accounts for 1–2% (Hussein *et al.*, 2011). Diagnosis of CBAVD is generally based on these criteria: the presence of normal- to slightly small-sized testicles, non-palpable vas deferens, normal plasma levels of FSH (follicle-stimulating hormone), and reduced ejaculate volume (<1 mL). Semen characteristics are as follows: azoospermic, acidic pH, undetectable or low fructose concentrations (normal: >25 μM) (Boucher *et al.*, 1999), α-glucosidase less than or equal to 5 mIU/ejaculate (normal: greater than or equal to 35 mIU/ejaculate) and carnitine less than or equal to 40 nM/ejaculate (normal: more than 260 nM/ejaculate) (Boucher *et al.*, 1999), and production of spermatozoa in the testicles.

When CBAVD is the only manifestation in a patient who harbors at least one mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, this condition is known as the genital form of cystic fibrosis (CF) (Anguiano *et al.*, 1992; Chillón *et al.*, 1995) and can be named CF-CBAVD. CF is an autosomal recessive genetic disease frequent in Euro-descendant populations, occurring in one of 2,000 newborns (Wainwright *et al.*, 1985; Boat *et al.*, 1989). Its incidence varies among different ethnic groups, with lower incidences in non-Euro-descendant populations. In Afro-descendants, the incidence varies from one in 14,000 to one in 17,000 newborns (Boat *et al.*, 1989; Fitzsimmons, 1993; Hamosh *et al.*, 1998), while in Asian populations, the frequency is one in 90,000 newborns (Boat *et al.*, 1989). The frequency of disease-causing mutation carriers is one in 20 in certain populations (Wainwright *et al.*, 1985).

Clinically, typical CF is characterized by chronic pulmonary obstruction, pancreatic insufficiency, high electrolyte concentration in sweat (‘salty sweat’) (White *et al.*, 1985), male infertility

(Mickle *et al.*, 2000), and other abnormalities. Kerem *et al.* (1989) state that 85% of CF patients have pancreatic insufficiency, although Noone & Knowles (2001) indicate that this prevalence is as high as 95%. Lung problems are the major cause of mortality and can be responsible for 95% of CF mortality (Boat *et al.*, 1989).

In 1985, Knowlton *et al.* (1985), White *et al.* (1985), and Wainwright *et al.* (1985) identified the location of the gene responsible for CF on the long arm of chromosome 7. Subsequent detailed studies have mapped the gene on 7q31.2. The main CF molecular defect was finally determined in 1989, when the *CFTR* gene was identified and cloned (Kerem *et al.*, 1989; Riordan *et al.*, 1989; Rommens *et al.*, 1989). Riordan *et al.* (1989) showed that it has a length of 250 kb and a total of 27 exons, numbered from 1 to 24, including exons 6a, 6b, 14a, 14b, 17a, and 17b.

The *CFTR* gene encodes a protein product of 1480 amino acids and molecular weight of 168,138 daltons (Riordan *et al.*, 1989). It is structured in five domains (Welsh & Smith, 1993): two membrane-spanning domains (MSD-1 and MSD-2) forming the channel and three cytoplasmic domains, from which two domains that bind to ATP (NBD-1 and NBD-2, *nucleotide-binding domain*) are connected by a regulatory domain R. These characteristics defined the name of the gene as *CFTR* (cystic fibrosis transmembrane conductance regulator).

Since its identification in 1989, more than 2000 mutations have been described in the *CFTR* gene (Cystic Fibrosis Mutation Database, CFMD, 2017). The most frequent mutation is p.F508del (c.1521_1523delCTT; rs113993960), with a worldwide frequency of 66% (Cystic Fibrosis Mutation Database, CFMD, 2017), varying among populations. This mutation consists of a three-nucleotide deletion in codon 508, resulting in loss of phenylalanine amino acid, which prevents the protein migration to the top of a plasma membrane (Kerem *et al.*, 1989).

Welsh & Smith (1993) propose four mutation classes in the *CFTR* gene: Class I consists of protein synthesis blocking mutations; class II represents changes in the protein processing; class III comprises changes in protein regulation; and class IV represents protein conductivity alteration. Wilschanski *et al.* (1995) added another class of mutation to the Welsh and Smith system, namely class V, leading to reduced protein synthesis. Mainly due to these different mutation classes, a broad range of phenotypes is seen in CF, varying from the typical manifestation to atypical forms including mild lung disease, idiopathic chronic pancreatitis, asthma, allergic bronchopulmonary aspergillosis, sinusitis, and the congenital bilateral absence of the vas deferens (CBAVD) (Noone & Knowles, 2001), which is the focus of this review.

Assisted reproductive techniques (ART) have enabled men with CBAVD to reproduce, and therefore, there is a need to identify and discuss the consequences of possible mutations in the *CFTR* gene in infertile couples.

Cystic fibrosis-related congenital bilateral absence of the vas deferens (CF-CBAVD)

CBAVD occurs in about 1–2% of infertile men (Hussein *et al.*, 2011), but in CF male patients, 95% have CBAVD as the result of mutations in the *CFTR* gene (Chillón *et al.*, 1995). There are cases of CBAVD that are not associated with CF, among them cases related to kidney malformations (Lane *et al.*, 2014). However, approximately 80–97% of patients that present with isolated CBAVD have a mutation in the *CFTR* gene (Casals *et al.*, 1995; Chillón *et al.*, 1995). Among these, 63–83% carry mutations in both alleles (Claustres *et al.*, 2000; Jézéquel *et al.*, 2000; Taulán *et al.*, 2007). Polymorphisms in other genes may increase the penetrance of CBAVD-related mutations. These include polymorphisms in the *CFTR* gene, such as certain polymorphisms in *Tr2GFB1* (transforming growth factor) and *EDNRA* (endothelin receptor type A) genes (Havasi *et al.*, 2010).

New or improved techniques for *CFTR* mutation screening have identified different mechanisms of mutations leading to CBAVD, as revealed by the identification of large rearrangements and deletions in the *CFTR* gene of CBAVD patients that could not be detected previously (Ratbi *et al.*, 2007; Taulán *et al.*, 2007; Trujillano *et al.*, 2013).

In general, mild phenotypes of CF (such as pancreatic insufficiency, mild lung problems, or atypical forms, such as CBAVD—Table 1) are caused by compound heterozygous genotypes with one severe mutation in one allele and one mild mutation in the other or, in some cases, one mild mutation in each allele (Chillón *et al.*, 1995; Cuppens *et al.*, 1998; Noone & Knowles, 2001). According to Uzun *et al.* (2005), one mild mutation in homozygous or two mild different mutations can cause atypical forms of CF or male infertility without any other clinical manifestation.

The reason the majority of men with CBAVD with two mutations in the *CFTR* gene do not present with lung problems is related to differences in the alternative mRNA splicing in different tissues (Cuppens & Cassiman, 2004). Studies by Mak *et al.* (1997) revealed that mRNA splicing was less efficient in the vas deferens epithelia than in the respiratory epithelia, an indication that the dysfunction of CFTR protein is more sensitive in the reproductive system than in other tissues. For example, in a patient homozygous for the IVS9-5T (c.1210-7_1210-6delTT variant, formerly known as IVS8-5T), a sequence of five thymines in

Table 1 Atypical (non-CF) diseases associated with the *CFTR* gene

Disease	Common manifestations shared with CF	Fraction of patients with at least one *CFTR* mutation	Reference
Allergic bronchopulmonary aspergillosis	Asthma, pulmonary infiltrates	Meta-analysis (26%)	Agarwal *et al.* (2012)
CBAVD	Absence of the vas deferens (bilateral)	Meta-analysis (78%)	Yu *et al.* (2012)
CUAVD	Absence of the vas deferens (unilateral)	9/24 (37.5%)	Casals *et al.* (2000)
Chronic pancreatitis	Abnormal pancreatic function	17/48 (35.4%)	De Cid *et al.* (2010)
Diffuse bronchiectasis (DB)	Abnormal dilation of bronchi	37/122 (30%)	Bienvenu *et al.* (2010)
Nasal polyposis	Nasal polyps	5/44 (11.4%)	Kostuch *et al.* (2005)
Neonatal transitory trypsinemia	High levels of immunoreactive trypsin	32/47 (62%)	Castellani *et al.* (2001)
Rheumatoid arthritis (RA)	Joint pain	5/24 (21%)	Puéchal *et al.* (2011)
RA and DB	Joint pain and abnormal dilatation of bronchi	18/30 (60%)	

intron 9 of the *CFTR* gene that results in loss of exon 10 leads to the formation of only 10% of the normal protein and causes malformation of the vas deferens. However, this is sufficient to prevent pathologies in other organs normally affected by CF (Chillón *et al.*, 1995; Mak *et al.*, 1997). Cuppens & Cassiman (2004) reported that the proportion of transcripts lacking *CFTR* exon 10 differs between vas deferens and nasal epithelium due to alternative splicing and to the presence of a mild mutation in the *CFTR* gene, with partial chloride channel activity, which causes dysfunction only in the vas deferens and not in the respiratory epithelium.

Mutations in the *CFTR* gene disrupt the function of the chloride channels, preventing them from regulating the flow of chloride ions and water across cell membranes. As a result, cells in the male genital tract produce mucus that is abnormally thick and sticky. This mucus clogs the vas deferens as it is forming, causing it to deteriorate before birth. The pathogenicity of CBAVD in CF may occur during development in utero, possibly by the obstruction of the genital tract due to accumulation of thick secretions that lead to degeneration of the vas deferens (Cuppens & Cassiman, 2004). Gaillard *et al.* (1997) observed the presence of the vas deferens in 12–18 weeks of aborted fetuses carrying a *CFTR* mutation, indicating that degeneration may occur later in embryonic development.

Although there are still several factors that remain unexplained in the etiology of CF-CBAVD, the main difference between typical CF and CF-CBAVD is the identification of different and rare *CFTR* mutations and variants in high frequency in individuals with CF-CBAVD as compared to the typical CF forms, such as the IVS9-5T (polymorphism Tn) variant, the (TG)m variant, the M470V (c.1408A>G, p.Met470Val) variant, and the high frequency of class IV and V *CFTR* mutations in CF-CBAVD cases.

IVS9-5T [c.1210-7_1210-6delTT; rs562195055; polymorphism Tn; formerly known as IVS8-5T]

The best characterized CBAVD-specific variant is the polymorphic polythymidine tract (Tn) in *CFTR* intron 9. The presence or absence of exon 10 in *CFTR* mRNA depends on the size of the sequence of thymines in intron 9 of the *CFTR* gene. This sequence, called poly-T, may contain 5, 7, or 9 thymines (T5, T7, or T9) and is generically known as c.1210_12T(5_9). The efficiency with which the splice acceptor site is used decreases in parallel with the size of poly-T chain (Chu *et al.*, 1993), which increases the probability of exon 10 loss during splicing and reduces the quantity of normal protein.

mRNA lacking exon 10 translates into an immature protein with no channel activity (Delaney *et al.*, 1993). A rare T3 allele (poly-T chain with three thymines) (Claustres, 2005) and recently a T2 allele (poly-T chain with two thymines) (Radpour *et al.*, 2009) have been associated with large losses of exon 10 during the splicing and can be considered mutations associated with CBAVD. Another example is the TGm allele (TG repeats immediately adjacent to the thymines in intron 9) that can alter the penetrance of Tn allele, more specifically the IVS9-5T (sequence of five thymines), being directly related to CF and CBAVD (Cuppens *et al.*, 1998).

About 10% of the world population carries the IVS9-5T (Kiesewetter *et al.*, 1993) allele. It presents as a pathogenic variant of incomplete penetrance (Cuppens *et al.*, 1998) with penetrance of 0.6, according to Zielenski *et al.* (1995), and is frequently encountered in men with CBAVD (a frequency of 40% was reported by Chillón *et al.*, 1995; and 25% by Mak *et al.*, 1999). The presence of the IVS9-5T variant in homozygote conditions produces about 95% of mRNA without exon 10 in the respiratory epithelium, resulting in an alteration in the NBD-1 domain of CFTR protein (Chu *et al.*, 1992).

A particular combination of two alleles (genotype) results in a certain level of mRNA which is normally produced and yields specific clinical phenotypes. A quantity of normally produced mRNA lower than 1–3% leads to a severe phenotype of CF. Levels of normal mRNA between 8% and 12% lead to a normal phenotype, and levels between 4% and 7% lead to atypical or mild forms of CF (Chillón *et al.*, 1995). Carriers of a typical *CFTR* mutation and an IVS9-5T allele may have low levels of normal mRNA, which is the most common cause of CBAVD (Chillón *et al.*, 1995). Osborne *et al.* (1994) reported that individuals with CBAVD may be homozygous or heterozygous for IVS9-5T, but must have a second *CFTR* mutation in *trans*.

In the work by Chillón *et al.* (1995), from 102 men with CBAVD, 33.3% had a *CFTR* mutation in one chromosome and a IVS9-5T allele in the other; 18.6% had two *CFTR* mutations that did not include the IVS9-5T; 19.6% had no IVS9-5T in both alleles but had a *CFTR* mutation in one chromosome; 6.9% had no *CFTR* mutation but one IVS9-5T allele; and 21.6% had no mutation detected (including IVS9-5T), indicating that another gene or genes may be related to CBAVD. Radpour *et al.* (2007) studied 112 Iranians with CBAVD and found 28.57% IVS9-5T alleles associated in *trans* with other mutations in the *CFTR* gene. The IVS9-5T allele and a p.F508del mutation were the most frequent causes of CBAVD in these patients, corresponding to more than one third of the identified alleles.

Bernardino *et al.* (2003), in a study with 20 Brazilian patients (17 with CBAVD and three with another type of obstructive azoospermia), found a frequency of the IVS9-5T allele in 23.5% of the men with CBAVD, similar to the one found in other studies (Chillón *et al.*, 1995; Casals *et al.*, 2000), which points out its relation with the CBAVD phenotype. Although individuals with an IVS9-5T allele in *trans* with a severe mutation in the *CFTR* gene show fertility problems (CBAVD) or other atypical forms of CF, approximately 40% are healthy and fertile due to the incomplete penetrance of this allele (Chillón *et al.*, 1995; Zielenski *et al.*, 1995).

In other populations, the frequency of the IVS9-5T in CBAVD patients varies between 3.1% in Mexico, suggesting that this mutation does not play a significant role in CF-CBAVD in that country (Saldaña-Alvarez *et al.*, 2012) and 45.6% in Italy (Giuliani *et al.*, 2010). In Algerian/Tunisian CBAVD patients, the IVS9-5T was found in 12.5% of the alleles (Boudaya *et al.*, 2012), but in China, IVS9-5T was found in 44.5% of the CBAVD alleles (Ni *et al.*, 2012). Similar frequencies for the IVS9-5T were found in Chinese (32.02%; Du *et al.*, 2014), Portuguese (31%; Grangeia *et al.*, 2007), Egyptian (30%; Hussein *et al.*, 2011), and Indian CBAVD men (27.1%; Sachdeva *et al.*, 2011).

(TG)m polymorphism

Repeats of 9 to 13 thymine–guanine (TG) downstream to the poly-T (Tn) sequence influence the exon 10 loss (Cuppens *et al.*, 1998; Niksic *et al.*, 1999). Unlike the Tn allele, which influences the efficiency of the splice acceptor site, the TGm alleles change the position of the splicing branch, as a larger number of TG repetitions increase the penetrance of the IVS9-5T allele and, consequently, the frequency with which the exon 10 is removed during splicing (Cuppens *et al.*, 1998). Jézéquel *et al.* (2000) found a frequency of 36.2% of IVS9-5T alleles in men with alterations in the vas deferens (including CBAVD). Among these, 52.9% were associated with a TG12 chain, 29.4% with a TG13 chain, and 17.7% associated with a TG11 chain.

Groman *et al.* (2004), in a study of 98 men with CBAVD, found nine men with other atypical forms of CF and 27 fertile men. They found the IVS9-5T allele in *cis* with three different TG repetitions: TG11-5T, TG12-5T, and TG13-5T. Among these, TG12-5T presented the stronger association with the pathogenesis (76% of the affected group). TG13-5T was found only in affected individuals. TG11-5T was considered generally benign as it was detected in 78% of the unaffected group.

Groman *et al.* (2004) concluded that when the IVS9-5T allele is in *trans* with a severe mutation, the pathogenicity is 28 and 34 times higher for TG12-5T and TG13-5T, respectively, than for TG11-5T. This allele combination implies a risk of 0.10 for TG11-5T, 0.78 for TG12-5T, and 1.0 for TG13-5T (Groman *et al.*, 2004). Radpour *et al.* (2007), in a study with 12 men with CBAVD, also found the TG12 and TG13 alleles associated in *cis* with the IVS9-5T allele.

Ni *et al.* (2012) found TG13 allele in a frequency 19-fold higher in Chinese CBAVD men (9.17%) than in controls (0.48%). The TG12 was significantly higher (55.05% CBAVD vs. 44.23% controls) and TG11 lower (35.78% CBAVD vs. 55.29% controls). The comparison of TG-T haplotypes revealed a significant 2.5-fold increase in the TG12-5T haplotype in men with CBAVD (33.94% vs. 13.46% in controls). TG11-5T and TG13-5T haplotypes were found 1.38% and 9.17%, respectively, in the CBAVD patients and were not found in the control group. However, significant increases in TG11-7T (55.29% controls vs. 34.4% CBAVD) and TG12-7T (30.29% controls vs. 21.1% CBAVD) haplotypes were observed in the control group. One case with the TG13-7T and TG12-9T genotype was found in the control group, which had not been reported previously. In summary, the IVS9-5T linked to either 12 or 13 TG repeats exhibits a high prevalence among the Chinese CBAVD patients tested. Therefore, the characterization of the TG chain size may indicate part of the penetrance of the IVS9-5T allele.

M470V (c.1408A>G; p.Met470Val; rs213950) variant

Cuppens *et al.* (1998) noticed an influence of the M470V allele in the penetrance of IVS9-5T. The polymorphic locus 470 (methionine or valine in the 470 codon) is located in exon 11 and codes part of the first NBD domain. Both 470 methionine (M470) and 470 valine (V470) lead to production of a CFTR completely glycosylated protein. Although M470 protein maturates more slowly than V470, M470 has a twofold increased chloride channel activity compared to V470 (Cuppens *et al.*, 1998).

Groman *et al.* (2004) reported that M470 is always associated with TG11-5T, and V470 with TG12-5T. The TG13-5T is exclusively found in those individuals affected by atypical CF forms (including CBAVD) and occurs only with M470. Du *et al.* (2014) and Ni *et al.* (2012) found no statistically significant difference between CBAVD and fertile men with regard to M470V genotype or allele frequencies. However, when the haplotype TG-T-M470V was considered, statistical analysis showed that the TG12-5T-V470 genotype was significantly associated with CBAVD (52.63%) as compared to normal controls (Ni *et al.*, 2012). Similarly, Stuppia *et al.* (2005) found a frequency of 84.6% of the haplotype TG12-5T-V470 in patients with CBAVD. According to Sun *et al.* (2006), 10 among 12 men affected with CBAVD carry this haplotype that has 80% penetrance in males. Pompei *et al.* (2006) showed that M470 allele presents higher variability in its adjacent areas, and many of these variations are mutations changing the constitution/function of the CFTR protein.

In another study, Ciminelli *et al.* (2007) analyzed the M470V locus in Italian couples requiring genetic counseling and found that in 39% of them, both partners had at least one M470 allele and 89% of these couples had an increased risk of having a child affected with CF. Based on that, a different screening for CF mutations should be performed in this subgroup. However, in a recent meta-analysis, Xu *et al.* (2014) found the variant M470V was a CBAVD protective factor among French, Chinese, Italian, and Iranian populations if this mutation is analyzed separately. This demonstrates the clinical and technical complexity needed to evaluate the relevance of a variant of a specific phenotype.

Other rare *CFTR* variants related to CBAVD

Only a few typical CF mutations, such as the p.F508del, are found in individuals with CF-CBAVD (Chillón *et al.*, 1995; Uzun *et al.*, 2005). Mak *et al.* (1999) reported that IVS9-5T is the most common variant among men with obstructive azoospermia followed by p.F508del, a finding supported by the analysis of these mutations in Portuguese men with CBAVD (31% IVS9-5T vs. 23.8% p.F508del) (Grangeia *et al.*, 2007).

The most frequent *CFTR* mutation is p.F508del, classified as class II, and therefore generally associated with the severe form of CF when in homozygosity or compound heterozygosity with a second 'severe' class I, II, or III allele. However, when associated with other 'mild mutations' (classes IV and V), or to specific variants such as the IVS9-5T (polymorphism Tn), the (TG)m, and the M470V, it can lead to atypical forms of CF, such as CF-CBAVD.

Classes IV and V *CFTR* mutations are strongly associated with the mild phenotypes of CF (Wilschanski *et al.*, 1995; Mak *et al.*, 1999). Among the class IV mutations, the R117H (p.Arg117His, c.350G>A) in combination with certain Tn alleles leads to different phenotypes: If associated with a IVS9-5T allele, generally it leads to CF; if in combination with a IVS9-7T, the allele can lead to mild forms of CF or to CBAVD (Kiesewetter *et al.*, 1993; Mak *et al.*, 1997; Noone & Knowles, 2001; Cuppens & Cassiman, 2004).

Jézéquel *et al.* (2000) reported that 19.1% of patients with vas deferens alterations had the 117 Arginine (R117) variation. Among these, 62.5% had the R117H_TG10_7T haplotype and 37.5% the R117H_TG11_7T haplotype. This is in agreement with the observation of Kiesewetter *et al.* (1993) who found men with malformation of the vas deferens and R117H mutation associated with allele IVS9-7T.

Thauvin-Robinet *et al.* (2013) reviewed the data from 179 non-newborn French individuals carrying R117H and a second *CFTR* variation. Among those, 76% were referred due to CBAVD. They concluded that patients with CBAVD carrying R117H and a severe CF variation should benefit from a clinical evaluation and follow-up and that depending on their genotype, a *CFTR* analysis should be considered in their partners to identify CF carrier couples and offer prenatal (PND) diagnosis or pre-implantation (PGD) diagnosis.

Other *CFTR* variants that have been found in CBAVD patients are shown in Table 2. The number of mutant alleles found in men with CAVD (congenital absence of the vas deferens) is summarized in Table 3.

Assisted reproductive techniques and genetic counseling

Most men with CBAVD are diagnosed with a mild form of CF only after the genetic cause of their infertility is identified (Martin *et al.*, 1992). Treatment of men with obstructive azoospermia (OA) as well as with CBAVD has not been available until the last three decades. Silber *et al.* (1990) was the first to report successful fertilization using epididymal spermatocytes, offering the possibility of men with CBAVD to have children. The technique was named MESA (microsurgical epididymal sperm aspiration) and consists of spermatocyte aspiration from the epididymis followed by *in vitro* fertilization. More recently, there

Table 2 *CFTR* mutations found in men with CBAVD from different nationalities/ancestries

Nationality/Ancestry	Mutation name	cDNA name	Protein name	Reference
Algerian	711+1G>T	c.579+1G>T	NA	Boudaya *et al.* (2012)
	E1104X	c.3310G>T	p.Glu1104X	
Asian	V201M	c.601G>A	p.Val201Met	Danziger *et al.* (2004)
	V520I	c.1558G>A	p.Val520Ile	
	Q1352H	c.4056G>C or c.4056G>T	p.Gln1352His	
Asian Indian	V456A	c.1367T>C	p.Val456Ala	
Chinese	1001+5G>A	NA	NA	Goh *et al.* (2007)
	870-1G-C	NA	NA	Li *et al.* (2012)
	1209+1G-C	NA	NA	
	1209+2T-G	NA	NA	
	3635delT	NA	NA	
	NA	NA	p.Ala357Thr	
	NA	NA	p.Thr388Lys	
	NA	NA	p.Arg419Ile	
Chinese	NA	NA	p.Gly451Lys	Li *et al.* (2012)
	NA	NA	p.Cys592Phe	
	M469V	c.1405A>G	p.Met469Val	Lu *et al.* (2013)
	S485C	c.1453A>T	p.Ser485Cys	
	E527N	NA	NA	
	I556V	c.1666A>G	p.Ile556Val	
	R553X	c.1657C>T	p.Arg553X	Lu *et al.* (2014)
	T501N	NA	NA	
	I507N	NA	NA	
	L558S	c.1673T>C	p.Leu558Ser	
French	L1227S	c.3680T>C	p.Leu1227Ser	Jézéquel *et al.* (2000)
	R117H	c.350G>A	p.Arg117His	Thauvin-Robinet *et al.* (2013)
Hispanic	W1098C	NA	NA	Danziger *et al.* (2004)
Indian	NA	c.650_659delAGTTGTTACA	p.Glu217Glyfs*11	Sachdeva *et al.* (2011)
	NA	c.3854 C>T	p.Ala1285Val	
Iranian	K536X	c.1606A>T	p.Lys536X	Radpour *et al.* (2006)
	Y122H	c.364T>C	p.Tyr122His	
Iranian	T338A	c.1012A>G	p.Thr338Ala	Radpour *et al.* (2006)
Italian	P499A	c.1495C>G	p.Pro499Ala	Arduino *et al.* (1998)
	D614G	NA	p.Asp614Gly	Tomaiuolo *et al.* (2011)
Japanese	A399D	c.1196C>A	p.Ala399Asp	Bernardino *et al.* (2003)
Jew	D1152H	NA	p.Asp1152His	Peleg *et al.* (2011)
Mexican	W1089X	NA	p.Trp1089X	Saldaña-Alvarez *et al.* (2012)
	G85E	NA	p.Gly85Glu	
Northern European Caucasian	P750L	c.2249C>T	p.Pro750Leu	Danziger *et al.* (2004)
Portuguese	P439S	c.1315C>T	p.Pro439Ser	Grangeia *et al.* (2007)
	V1108L	c.3322G>C	p.Val1108Leu	
	P1290S	c.3868C>T	p.Pro1290Ser	
	E1401K	c.4201G>A	p.Glu1401Lys	
Spanish	DeltaE115	c.343_345del	p.Glu115del	Casals *et al.* (1995)
	K1060T	c.3179A>C	p.Lys1060Thr	
Taiwanese	N287K	NA	NA	Wu *et al.* (2005)
	M469I	NA	NA	
	S895N	NA	NA	
United States	R766M	c.2297G>T	p.Arg766Met	Ravnik-Glavac *et al.* (2000)
	R792G	c.2374C>G	p.Arg792Gly	
	G542X	c.1624G>T	p.Gly542X	Wang *et al.* (2002)
	G551D	c.1652G>A	p.Gly551Asp	
	R334W	c.1000C>T	p.Arg334Trp	
	W1282X	c.3846G>A	p.Trp1282X	
	N1303K	c.3909C>G	p.Asn1303Lys	
Various	A800G	c.2399C>G	p.Ala800Gly	Mercier *et al.* (1995)
	G149R	c.445G>A	p.Gly149Arg	
	R258G	c.772A>G	p.Arg258Gly	

NA, not attributed.

Table 3 Percentage of abnormal alleles detected in men with CAVD

Number of mutant *CFTR* alleles Other than 5T	5T	%
2	0	26
0	2	2
1	1	26
1	0	17
0	1	8
0	0	22

Reference: Moskowitz *et al.* (2001).

are other techniques to obtain spermatocytes from individuals with OA, such as PESA (percutaneous epididymal sperm aspiration), FNA (fine needle sperm aspiration), and TESA (testicular sperm aspiration). The fertilization is made by ICSI (intracytoplasmic sperm injection) (De Kretser & Baker, 1999).

Kamal *et al.* (2010), in an *in vitro* fertilization program using ICSI found similar rates of fertilization, clinical pregnancy, and miscarriage between men with CBAVD and patients having other causes of OA. Attardo *et al.* (2001) found a pregnancy rate of 30% (and fertilization rate around 50.7%) for men with CBAVD, a rate similar to the one obtained for non-CBAVD infertile men. This suggests that mutations on the *CFTR* gene do not alter the potential of spermatozoa fertilization. However, recent studies have demonstrated that CFTR protein is involved in a number of processes. These include spermatogenesis and sperm capacitation, acting not only as a simple ion-conducting channel but also as a regulator of other channels/transporters through protein–protein interactions and mediating the activation or inhibition of different signaling pathways, including sAC/cAMP/PKA and NF-kB/COX-2/PGE2, leading to alterations in transcriptional activities important for various reproductive processes (Chen *et al.*, 2012). These are possible molecular mechanisms underlying the clinically observed link between *CFTR* mutations and male infertility other than CBAVD (Chen *et al.*, 2012).

In accordance with Attardo *et al.* (2001), Lu *et al.* (2014) found similar rates of fertilization (70.1% and 68.2%, respectively), embryo quality (51.1% and 52.1%), clinical pregnancy (49.7% and 48.8%), and ectopic pregnancy (5.7% and 2.6%) between CBAVD and non-CBAVD patients who had PESA followed by ICSI. However, the rate of miscarriage/stillbirth (death before or after 20 weeks of gestation, respectively) was higher in men with CBAVD (23.9%) than in those with non-CBAVD obstruction (12.5%, $p < 0.001$). The rate of live births was lower in men with CBAVD (70.5%) than in those with non-CBAVD obstruction (84.9%, $p < 0.001$). Thus, patients with CBAVD presented a significantly increased risk of miscarriage or stillbirth. This risk is possibly a result of *CFTR* mutations, as the frequency of *CFTR* mutations was threefold higher in the CBAVD group (13.0%) than that in the non-CBAVD group (4.1%, $p < 0.001$) (Lu *et al.*, 2014).

Besides the fertilization, miscarriage/stillbirth rates, and the role of the *CFTR* gene in male infertility, *CFTR* mutations from CBAVD patients submitted to ART can be transmitted to offspring. Mak *et al.* (1999) discuss the difficulty to predict phenotypic characteristics for a child carrier of one or other inherited allele, until the genotypic–phenotypic correlations of *CFTR* mutations are fully understood. Mak *et al.* (1999), Danziger *et al.* (2004), and Wong *et al.* (2004) underscore the importance of complete sequencing of the *CFTR* gene in men with CBAVD who desire to have children with ART.

The large number of *CFTR* variants already detected and the fact that only a few of them have been proven to be pathogenic suggest that genetic counseling and testing in CF should be carried out by specialized reference centers. Recently, the CFTR2 Consortium showed that the M470V variant cannot be considered a pathogenic mutation (The Clinical and Functional Translation of CFTR, CFTR2, 2015).

Data from the CFTR2 Consortium also indicated that children diagnosed with CF (through newborn screening) but carrying non-CF-causing variants in one allele and one CF-causing variant in the other allele have significantly higher birthweight and first-year growth rate and lower immunoreactive trypsinogen and sweat chloride values, as well as lower rate of persistent *Pseudomonas aeruginosa* colonization when compared to children with two CF-causing variants (Salinas *et al.*, 2015).

De Kretser & Baker (1999) suggest that genetic analysis should start with the female partner of men with CBAVD. If a comprehensive *CFTR* analysis is performed and no mutations in the *CFTR* gene are found, then the risk that a child develops any pathology due to mutations in this gene is reduced, and the genetic analysis in the male partner is not 100% required, thereby reducing the costs of genetic analysis.

Stuppia *et al.* (2005) reported that the common mutations in *CFTR* gene in patients who undergo ART are not found at a rate higher than those expected for the general population, and the risk for a couple of having a child with CF is considerably reduced, as long as there is no previous family history of the disease. This is in accordance with the suggestion from De Kretser & Baker (1999) that the genetic analysis has to be performed for one of the parents, preferably in the infertile one, and if a mutation is found in the *CFTR* gene or an IVS9-5T allele, then genetic analysis should be performed in the other parent. Additionally, they consider that the analysis of the association between loci TG-5T-M470V can help with the risk calculation of having a child affected by the mild form of CF or CBAVD in couples in which one partner carries mutation in the *CFTR* gene and the other is a carrier of IVS9-5T allele.

Conversely, Mak *et al.* (1999) emphasize the importance of performing genetic analysis in both male and female partners, as the relation between genotype and phenotype is not well established, and the consequences of inheriting at least one mutation are unknown. Attardo *et al.* (2001) suggest that men with CBAVD should be considered carriers of at least one mutation in the *CFTR* gene, unless the entire gene is analyzed and mutations are ruled out.

Cuppens & Cassiman (2004) explain that a man with CBAVD who carries one severe mutation in the *CFTR* gene has a 50% probability of transmitting this mutation to offspring. The probability that his female partner is a carrier of a mutation in the *CFTR* gene is one in 20, and the transmission of this mutation is 50%. For this couple, the risk of having a child with CF is one in 100—a risk 25 times higher than for the general population (one in 2500). When no mutation is detected in the female partner, the risk for the couple is one in 1000 (2.5 times higher than for the general population) (Cuppens & Cassiman, 2004).

For Euro-Brazilians, the frequency of mutation within *CFTR* gene is one in 44 (Raskin *et al.*, 2008). If the male partner has CBAVD due to a *CFTR* gene mutation, the risk for a couple to have a CF child is approximately 5.7 in 1000—a risk 43 times higher than for the general population (one in 7576).

Therefore, genetic counseling for couples in which the male partner has CBAVD is very important to estimate the risks and possible genotype–phenotype correlations. In addition to genetic counseling, diagnosis for embryo implantation in the uterus (pre-implantation genetic diagnosis—PGD) or prenatal diagnosis (Mak *et al.*, 1999; Crosignani & Rubin, 2000; Viville *et al.*, 2000; Allen *et al.*, 2006) can be performed routinely. An informed consent should be obtained from the couple before initiating any ART.

CONCLUSION

CBAVD can be a form of atypical CF and leads to infertility in the majority of male carriers of *CFTR* gene mutations. With ART widely available, men with CBAVD are able to reproduce. This increases the risk of passing on deleterious genes to descendants. Thus, every ART specialist should investigate whether azoospermia is due to CBAVD. If so, and CBAVD is due to *CFTR* mutations, the infertile couple should be informed about the reproduction consequences before ART. Couples at risk should be offered comprehensive *CFTR* genetic testing, taking into account the ethnic group of the patient and subsequent counseling. When a couple seeks ART, their obvious and main goal was to achieve pregnancy and eventually have a child. However, the first goal of the physician should be to identify the cause of infertility, if possible, and also to offer every available technique, such as pre-implantation genetic diagnosis, to minimize the risk of having an affected child with chronic or severe disease.

ACKNOWLEDGEMENTS

We thank Diane Cooper, MSLS, NIH Library, for providing assistance in writing this manuscript. This research was supported in part by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health (NIH); and in part by a grant from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Process: 311166/2011-3 - PQ-2 (to F.R.F.).

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