Review Article

CFTR Mutations in Congenital Absence of Vas Deferens

Ramin Radpour, M.Sc.[1*], Iman Salahshourifar, M.Sc.[1], Hamid Gourabi, Ph.D.[1],
Mohamad Ali Sadighi Gilani, Ph.D.[2], Ahmad Vosough Dizaj, M.D.[2]

1. Reproductive Genetics Department, Royan Institute
2. Andrology Department, Royan Institute

Abstract

A qualitative diagnosis of infertility requires attention to female and male physical abnormalities, endocrine anomalies and genetic conditions that interfere with reproduction. Many genes are likely to be involved in the complex process of reproduction. Cystic fibrosis (CF) incidence varies in different White people populations (a higher incidence of CF is observed in northern–western European populations than in southern European populations), and therefore the incidence of congenital bilateral absence of the vas deferens (CBAVD) may also vary in different White people populations. As CF is mainly observed in White people, hardly any data are available of CBAVD in non-White people, but frequent polymorphisms such as *5T* are observed in most populations. The spectrum and distribution of cystic fibrosis transmembrane conductance regulator gene (*CFTR*) mutations differs between CBAVD and CF patients, and even compared with control individuals. Combinations of particular alleles at several polymorphic loci yield insufficient functional CFTR. The combination of the *5T* allele in one copy of the *CFTR* gene with a cystic fibrosis mutation in the other copy is the most common cause of CBAVD in Iran. Because of techniques such as intracytoplasmic sperm injection (ICSI), CBAVD patients are now able to father children, however such couples have an increased risk of having a child with cystic fibrosis, and therefore genetic testing and counseling should be provided. Around 10% of obstructive azoospermia is congenital and is due to mutations the CF gene. This paper reviews the relationship of mutations in the *CFTR* gene with CBAVD.

Keywords: Cystic Fibrosis Transmembrane Regulator (*CFTR*), Congenital Bilateral Absence of Vas deferens (CBAVD).

Introduction

A qualitative diagnosis of infertility requires attention to female and male physical abnormalities, endocrine anomalies (their hormonal and genetic background) and genetic conditions that interfere with reproduction. Many genes are likely to be involved in the complex process of reproduction (1). Genetic and non-genetic causes of infertility are increasingly being identified. Infertility, or at least subfertility, in males with cystic fibrosis, was first suspected in the 1960s (2). Infertility in males with CF typically originates in developmental abnormalities in the vas deferens or the distal half of the epididymis. In 97–98% of men with CF, a bilateral congenital absence of the vas deferens (CBAVD) blocks the transport of spermatozoa from testicular or epididymal structures to the outer genital tract, resulting in azoospermia (3).

Females with CF are found to be less fertile than normal healthy women. In CF females delayed puberty and amenorrhoea are common due to malnutrition as the main cause (3).

CFTR gene mutations and polymorphisms

The main genetic causes of male infertility are microdeletions of the Y chromosome (AZF region) connected with oligospermia or azoospermia as well as mutations of cystic fibrosis transmembrane conductance regulator (*CFTR*) gene which is connected with azoospermia and congenital bilateral absence of vas deferens (CBAVD) (4).

The *CFTR* gene spans about 190 kb at the genomic level and contains 27 exons.

Several alternatively spliced transcripts have been found, the most important one that lacks exon 9 sequences (5).

The CFTR protein is a glycosylated transmembrane protein, which functions as a chloride channel.

CFTR is expressed in epithelial cells of exocrine tissues, such as the lungs, pancreas, sweat glands and vas deferens. Apart from its chloride channel function it also functions as a regulator of, and is regulated by, other proteins (6). More than 1500 CF-causing *CFTR* mutations have been identified (Table 1) (http://genet.sickkids.on.ca/).

Table 1: Frequency of different types of CFTR gene mutations in CF and CBAVD patients (http://genet.sickkids.on.ca/).

Mutation Type	Count	Frequency (%)
Missense	643	41.81
Frameshift	242	15.73
Splicing	196	12.74
Nonsense	149	9.69
In frame in/del	31	2.02
Large in/del	42	2.73
Promotor	8	0.52
Sequence variation	225	14.63

Most mutations are point mutations, i.e. only one nucleotide is mutated in a *CFTR* gene. A CF patient can either carry an identical *CFTR* mutation on both *CFTR* alleles, or two different *CFTR* mutations on both *CFTR* alleles and is then called compound heterozygous for two *CFTR* mutations. The distribution of *CFTR* mutations differs between different ethnic populations. The most common mutation, F508del, reaches frequencies of about 70% in Northern European populations, while lower frequencies are observed in Southern European populations. Besides F508del, other common mutations exist in most populations, each reaching frequencies of about 1–2%. Examples include the G542X, G551D, R553X, W1282X and N1303K mutations. Finally, for a given population, ethnic-specific mutations that reach frequencies of about 1–2% might exist. For most populations, all these common mutations cover about 85–95% of all mutant *CFTR* genes. The remaining group of mutant *CFTR* genes in a particular population comprises rare mutations, some of them only found in a single family.

In CBAVD patients having two mutant *CFTR* alleles, at least one will be a mild mutation. In the CBAVD patients in which a mutation is found on both *CFTR* genes, about 88% of them carry one severe mutation on one *CFTR* gene and a mild mutation on the second *CFTR* gene, and about 12% carry mild mutations on both *CFTR* genes (7). This in contrast to CF, were about 88% of the CF patients carry severe mutations on both *CFTR* genes and about 11% carry a severe mutation on one *CFTR* gene and a mild mutation on their second *CFTR* gene (7). The most frequent *CFTR* mutation conferring a mild phenotype found in CBAVD patients is the *5T* polymorphism (8). In the White people population, the *5T* polymorphism is found on about 21% of the *CFTR* genes derived from CBAVD patients, while it is only found on about 5% of the *CFTR* genes derived from control individuals. *5T* is one of the alleles found at the polymorphic Tn locus in intron 8 of the *CFTR* gene. Either a stretch of 5, 7, or 9 thymidine residues is found at this locus, hence the alleles *5T*, *7T* and *9T*. A less efficient splicing will occur when a lower number of thymidines are found (Fig 1), resulting in *CFTR* transcripts that lack exon 9 sequences (5).

Alternatively spliced *CFTR* transcripts lacking exon 9 sequences are found in any individual, but the extent varies depending on the alleles present at the Tn locus. In individuals homozygous for a *5T* allele, up to 90% of the *CFTR* transcripts lack exon 9 (5). *CFTR* transcripts that lack exon 9 sequences result in CFTR proteins that do not mature (9, 10). When *5T* is found in compound heterozygosity with a severe *CFTR* mutation, or even *5T*, pathology might be observed such as CBAVD. However, not all male individuals that are compound heterozygous for a severe *CFTR*

mutation and *5T* develop CBAVD, such as some fathers of CF children. The *5T* polymorphism was therefore classified as a disease mutation with partial penetrance. We found that the partial penetrance can be explained by another genetic factor, namely the polymorphic TGm locus in front of the Tn locus (11). Different alleles can be found depending on the number of TG repeats found at this locus. The higher number of TG repeats, the less efficient exon 9 splicing will occur (Fig 2). The *5T* polymorphism can be either found in combination with a TG11, TG12 or TG13 allele (respectively 11, 12 or 13 TG repeats). In CBAVD patients, the milder TG11-*5T* allele is hardly found, while the TG12-*5T* is most frequently found. The TG13-*5T* is rarer, but also found in CBAVD patients. TG13-*5T* might even result in pancreatic sufficient CF, possibly because of additional polymorphisms that affect *CFTR* such as V470 (11). In individuals that are compound heterozygous for a severe mutation and the *5T* allele, such as fathers of CF patients, *5T* is associated with the milder TG11 allele.

Depending on the effect at the protein level, *CFTR* mutations can be divided in at least five classes (12). Class I mutations result in no CFTR synthesis, because of mutations affecting splice sites, nonsense mutations resulting in truncated CFTR protein which are mostly unstable and therefore degraded, and mutations shifting the coding frame in the gene (frameshift deletions and insertions). Class II mutations, such as the most common mutation F508del, result in CFTR proteins that fail to mature and that are degraded. Class III mutations result in CFTR proteins that mature, and therefore reach the apical membrane of the cell, but which result in abnormal regulatory properties of the chloride channel. Class IV mutations result in CFTR channels having abnormal conductive properties, because of mutations in the conductivity pore. Finally, class V mutations result in some functional CFTR proteins. Class I, II and III mutations

are severe mutations, while class IV and V mutations are known as mild mutations.

CFTR protein and molecular determinants of the channel pore

The CFTR molecule is made up of two homologous repeats, each containing six transmembrane (TM) regions followed by an intracellular nucleotide binding domain (NBD; Fig 2).

These two halves are joined by an intracellular regulatory or R domain. Recently a low resolution crystal structure of CFTR was obtained (13), which showed membrane spanning regions lining a central pore; the pathway through which Cl-ions cross the membrane. However, the identity of the TM regions forming the pore, or even the number of TMs that line the pore, cannot be identified in this structure. Nevertheless, homology with the structures of other ATP-binding cassette (ABC) proteins (13, 14) suggests that the CFTR pore is lined by multiple α-helical TM regions in a reasonably parallel fashion. This overall pore architecture is common with ligand-gated Cl−channels (15) but in stark contrast with the seemingly haphazard arrangement of membrane associated α-helices observed in Cl$^-$ channels (16).
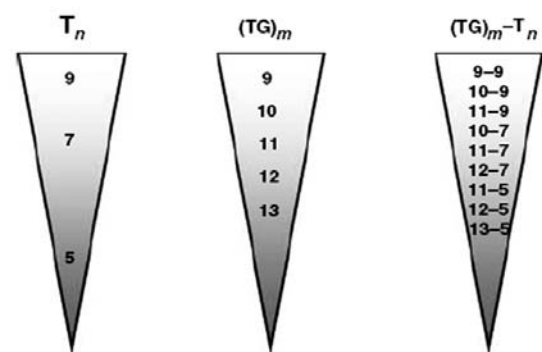

Figure 1: The effect of particular alleles on the amount of functional CFTR. For different polymorphic loci (Tn and TGm) or haplotypes (TGm-Tn), the effect of each allele/haplotype on the amount of CFTR chloride channel activity is shown. Decreasing amounts of functional CFTR are obtained from top to bottom.

CFTR gene and development of the vas deferens

Given the fact that *CFTR* mutations are detected in men with CBAVD and, with a lower frequency, in men with unilateral absence of vas deferens (CUAVD), it appears that a normal amount of a functional CFTR protein is required to ensure proper development of the vas deferens. However, the lack of *CFTR* mutations detection in CBAVD with renal abnormalities also suggests that the mesonephric duct plays an important role in the development of the vas deferens. Therefore, it can hypothesize that the CFTR protein is required at some specific embryonic stage of the development of the vas deferens. The mechanism(s) by which *CFTR* mutations contribute to the pathogenesis of CBAVD is (are) still unknown.
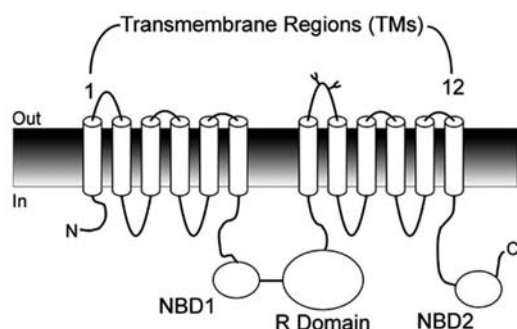

Figure 2: Topology of CFTR protein. CFTR comprises 12 TM regions (organized into two groups of six), two intracellular NBDs and the intracellular R domain.

Genotype-phenotype correlations in cystic fibrosis

Analyses of the correlation between phenotype and genotype showed that the *CFTR* mutations could be grouped in two categories, mild or severe, with respect to pancreatic function (17).

The severe mutations are associated with pancreatic insufficiency whereas the mild mutations, leading to a higher residual CFTR activity, confer pancreatic sufficiency (17).

A CF patient is likely to be pancreatic sufficient if he has one or two mild mutations. Several studies have shown that some genotypes are associated with a severe phenotype, while others have a milder clinical impact and evolution.

The development of molecular techniques to identify *CFTR* mutations now allows testing of this hypothesis, which was done in several studies over the past few years.

Discordant familial segregation of alleles in patients with CBAVD

Familial studies are another approach to study the possibility of genetic heterogeneity. In families with CBAVD linked to *CFTR* mutations, brothers with CBAVD should inherit the same *CFTR* genes from their parents, whereas fertile male siblings should have at least one *CFTR* gene different from their CBAVD brothers.
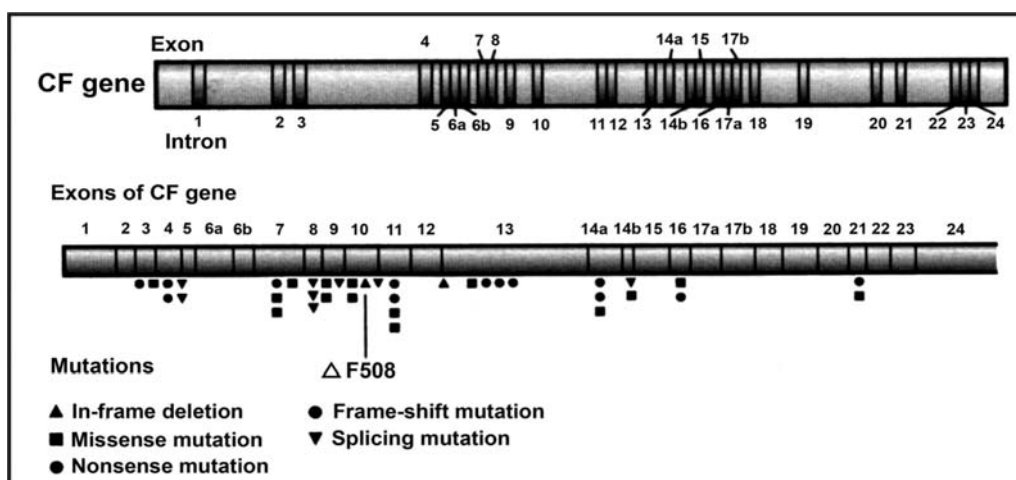

Figure 3: *CFTR* mutation spectrum of Iranian CBAVD patients: this picture shows the panel of 19 different mutations with their frequency in our studied population (Radpour et al. 2007).

In families with CBAVD not linked to CFTR defects, the inheritance of the *CFTR* genes should be random with respect to the CBAVD phenotype.

Familial segregation of *CFTR* genes can be assessed by studying polymorphisms within and around the *CFTR* gene: the combination of alleles at the polymorphic sites (haplotypes) allows one to follow transmission of *CFTR* genes from parents to children in the pedigrees.

Three families with no identified *CFTR* mutations have been reported in which either the brothers with CBAVD inherited two different alleles from one of their parents (18) or in which fertile brothers inherited the same *CFTR* alleles as their brothers with CBAVD. Such discordance between phenotypes and marker haplotypes suggested that CBAVD was not caused by two mutated *CFTR* alleles but was rather the result of mutations at other hypothetic loci (18).

However, such cases could also be explained by *CFTR* unidentified mutations that have a more complex pattern of penetrance, and it would have been interesting to re-analyze these three families with the *5T* allele and its surrounding modifiers.

Moreover, whether CBAVD was associated with renal anomalies, or not, was not mentioned in the initial reports. Nevertheless, 15–20% of patients with CBAVD who have now been extensively screened and do not have *CFTR* mutations or the *5T* allele may indicate a subpopulation of men with other genes responsible for the reproductive anomaly.

Males with *CFTR* mutations (CBAVD males)

Majority of adult males with CF (99%) has CBAVD. CBAVD is also encountered in 1-2% of infertile males without cystic fibrosis (19). Men with CBAVD but without CF gene mutations have a high incidence of urinary tract malformations (19). The group with urinary tract anomalies represents a separate clinical entity not related to CF and with different embryological pathogenesis. Some forms of infertility found in otherwise healthy men have also been reported to be associated with *CFTR* mutations, especially obstructive azoospermic conditions such as congenital CBAVD, CUAVD, epididymal obstruction and bilateral ejaculatory duct obstruction with concomitant seminal vesicle anomalies (50).

CBAVD is caused by a disruption in the vas deferens, a Wolffian duct derivative. In a widespread study of CBAVD in Iran, among 120 cases of men with CBAVD, The analysis of the entire coding sequences of *CFTR* gene, allowed us to identify 19 different mutations in Iranian CBAVD patients (Fig 3).

These mutations have been described previously in Iranian patients with CBAVD (20, 21). Of those, 5 cases were homozygous or compound heterozygous (+/+), 67 were positives for only one mutations (+/-) and 49 cases were negative for both mutations (-/-) (IVS8-5T was not involved). The result of our study reflects the high allelic heterogeneity of *CFTR* gene mutations, although two mutations, IVS8-*5T* and F508del, were found to be more common in Iranian CBAVD patients. IVS8-*5T* was observed with TG12 or TG13 haplotypes, on 61 chromosomes thus confirming the association of this splice site variant with CBAVD in Iranian patients.

Screening for the IVS8-*5T* and F508del together led to the identification more than one-third of alleles. All of patients with completely resolved mutation genotype carried a missense or splicing mutation on at least one allele but in three cases we could find one nonsense mutation (22). The diagnosis of CBAVD is based on the presence of azoospermia in subjects with normal or small size testes, non-palpable vas deferens, the characteristic ultrasonography view and changes in the physical and biochemical properties of ejaculate (small volume, low pH, and low fructose concentration; (19). Genital abnormalities

may occur early in CF, but are less common diagnosed than in adults. They are found more often in pancreatic insufficient than in pancreatic sufficient CF patients (8).

Mutations in the cystic fibrosis transmembrane conductance regulator gene are a relatively frequent cause of male infertility. Depending on their molecular consequences, *CFTR* mutations may either result in a typical cystic fibrosis, or in an atypical (often monosymptomatic) forms of CF such as congenital absence of the vas deferens (bi- or unilateral), bilateral ejaculatory duct obstruction or bilateral obstructions within the epididymides. Infertile men might have an elevated risk of being carriers of a mutation within the *CFTR* gene, in comparison with the general population. Therefore, it is recommended to conduct *CFTR* mutation tests in males with idiopathic infertility prior to reproduction techniques.

Additionally, Chu et al. have identified the mutations within the polypyrimidine tract of the *CFTR* intron 8-splice acceptor site (IVS8-T tract), consisting of five, seven, nine thymidines (*5T*, *7T*, *9T*). The *5T* variant produces a lower level of normal *CFTR* mRNA transcripts than the *7T* and *9T* alleles and is associated with disseminated bronchiectasis, CBAVD and epididymal obstruction (5). Arduino et al. have described a CBAVD patient with a compound heterozygosity in the *CFTR* gene for a stop mutation W1282X and a new missense mutation P499A. The P499A was found as a mild mutation, limited to the development of Wolffian duct derivatives and was revealed only in combination with a severe *CFTR* mutation (23).

More recently, it was suggested that the frequency of CF mutations in infertile males was significantly higher than the expected CF carrier frequency in the general population (3).

This increased frequency in healthy men with reduced sperm quality and in men with azoospermia without CBAVD suggested that the CFTR protein could be involved in the process of spermatogenesis or sperm maturation apart from playing a critical role in the development of the epididymal glands, the vas deferens and seminal vesicles.

The use of percutaneous epididymal or testicular sperm aspiration (PESA, TESA) and intracytoplasmic sperm injection (ICSI) has allowed the attainment of pregnancies in this group of patients (Silber). If any CF mutation is detected in a patient, *CFTR* screening should involve the female partner. The female partner of a man with CFTR-mediated anomalies of the genital tract may be heterozygous purely by chance (4–5% in many countries).

Since infertility in many cases can be treated nowadays by assisted reproductive technology, there is an increased risk of having children with CF if the male and female are carriers of *CFTR* mutations.

Couples should be informed about the risk and risk prevention including pre-implantation diagnosis. Follow-up studies of children born to these couples are mandatory, whether male infertility is linked to CF or not (24).

Up to 20% of the CBAVD patients, absence of the vas deferens is associated with renal malformations.

In a small study, this CBAVD etiology was suggested not to be related to *CFTR* mutations, as no *CFTR* mutation could be identified in this group of 10 CBAVD patients (25).

However, in a more recent study, two of four CBAVD patients with only one kidney were carrier of a *CFTR* mutation, one of them even compound heterozygous for F508del and *5T* (26).

Involvement of *CFTR* in other forms of male infertility than CBAVD

There are reports that *CFTR* is also involved in other forms of infertility than CBAVD. In a small study of 17 patients having obstructive azoospermia in which the vas deferens and/or epididymis is present but obstructed, a mutation in the *CFTR* gene was identified in eight of 34 (23.5%) *CFTR*

genes, from these patients (27). It should be noted that five of the eight mutant *CFTR* genes carried the *5T* allele.

In another study, 14 out of 80 (17.5%) men with a variety of diagnoses varying from oligozoospermia to oligoasthenoteratozoospermia carried a *CFTR* mutation (3).

However, in another report were 75 patients with oligoasthenoteratozoospermia were studied, the frequency of *CFTR* mutations was not significantly different from the control population (28).

The involvement of *CFTR* in other forms of male infertility than CBAVD is thus not clear and needs to be further investigated. Indeed, *CFTR* is regulated during the generation of spermatozoa in rats (29).

Here, *CFTR* transcripts are confined to post-meiotic round spermatids. During this development stage, haploid spermatids are converted into spermatozoa.

Nucleus condensation and decrease in cytoplasm volume, which is thought to be caused by reduction of intracellular water content, occur in this phase.

Maximal *CFTR* expression precedes this stage.

It can therefore not be excluded that very mild functional *CFTR* polymorphisms are still involved in other forms of male infertility than CBAVD.

Assisted reproduction in CBAVD couples and the risk of having CF children

Because of techniques such as ICSI, CBAVD patients nowadays are able to father children. However, if sperm cells of a CBAVD patient are used in an ICSI program, there is an increased risk of having a child with CF. Indeed, most CBAVD patients carry a severe CF-causing *CFTR* mutation, and therefore have a chance of 0.5% of transmitting the CF-causing *CFTR* mutation to the child.

Assuming a risk of 1/25 of the partner being a CF carrier, and when a carrier, again a chance of 0.5% of transmitting the mutant *CFTR* gene to the child, the combined risk of CBAVD couples of having a CF child is 1 in 100 (compared with a risk of 1/2500 in the control population).

Male with CBAVD	Female partner	Offspring genotype	Offspring Phenotypes (estimated risks)	
1 severe/1 mild	1 severe/1 normal	1/4 severe/severe	➡ 25%	*CF-PI*
		1/4 severe/normal or 1/4 mild/normal	➡ 50%	no disease
		1/4 mild/severe	➡ 25%	CBAVD, *CF-PS*, no disease, other ?
			risk *of CF = 1/2*	
1 severe/1 mild)	negative (detection rate 90%) (Z =1/241)	1/4 severe/Z	➡ 25%	Z/4
		1/4 severe/normal or 1/4 mild/normal	➡ 50%	normal phenotype
		1/4 mild/Z	➡ 25%	Z/4 x penetrance
			risk of CF = *1/964*	

Figure 4: Risk calculation and phenotype prediction in males with CBAVD (risk is calculated with an assumption of a CF carrier frequency of 1 in 25) (Claustres, 2005).

Genetic counseling should therefore be provided to such CBAVD couples and the partner should be tested for *CFTR* mutations in order to determine the risk of having a CF child. However, given the fact that most *CFTR* genetic tests only analyze the most common CF-causing *CFTR* mutations, the carrier status of the partner may remain undetected.

Assuming that genetic tests most routinely used have a sensitivity of about 90%, when no mutation is found with the test, the partner still has a risk of being a carrier of 1/250 and therefore the CBAVD couple has still a risk of 1/1000 of having a CF child.

It should be noted that most genetic tests for *CFTR* mutations, especially commercial tests, screen only for the most frequent CF causing mutations, and not the milder rare mutations, therefore resulting in a *CFTR* mutation detection rate of only about 60% in CBAVD patients (30).

If the male partner proves to be a carrier of one of the eight CF mutations routinely tested for, then it is an obvious requirement to screen the female partner. If the female partner proves to be negative for these mutations then the risk of an affected child would be 1:960 and treatment could proceed with the appropriate degree of counseling.

Risk of CF, CBAVD or CFTR-opathies for offspring

For a couple with CBAVD associated with CFTR defects, planning to have their own genetic children, the risk for both male and female offspring to have CF or related diseases and for male offspring to have CBAVD depends on whether or not the female partner is a carrier, since one mutated allele will always be inherited from the male. As the carrier frequency of *CFTR* mutations in many Caucasian populations is in the order of 1/22–1/30, it is highly recommended that genetic testing for *CFTR* mutations be offered to the couple prior to ICSI. The genetic counsellor should determine whether there is a family history of CF and the couple's ethnicity, as this will affect their carrier risk.

The genetic of CBAVD is more complex than in CF, as (i) genetic analysis is able to prove but not to exclude the diagnosis of a genital form of CF, and (ii) the risk of CF or CBAVD in the offspring may be unpredictable when rare mutations are identified in the male or the female. The couple should be informed that the test cannot detect all mutations within the gene; therefore, a negative mutation screen reduces, but does not eliminate, the risk of being a carrier (Fig 4).

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