Mutations of the cystic fibrosis transmembrane conductance regulator gene in males with congenital bilateral absence of the vas deferens: Reproductive implications and genetic counseling (Review)

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Abstract. Congenital bilateral absence of the vas deferens (CBAVD) is predominantly caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CBAVD accounts for 2-6% of male infertility cases and up to 25% of cases of obstructive azoospermia. With the use of pre-implantation genetic diagnosis, testicular or epididymal sperm aspiration, intracytoplasmic sperm injection and *in vitro* fertilization, patients affected by CBAVD are able to have children who do not carry CFTR gene mutations, thereby preventing disease. Therefore, genetic counseling should be provided to couples receiving assisted reproductive techniques to discuss the impact of CFTR gene mutations on reproductive health. In the present article, the current literature concerning the CFTR gene and its association with CBAVD is reviewed.

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1. Introduction

Congenital bilateral absence of the vas deferens (CBAVD) accounts for 2-6% of male infertility cases and up to 25% of cases of obstructive azoospermia (1,2). In addition to CBAVD, the clinical symptoms and characteristics of this condition generally include normal or slightly small testicles, atrophy or absence of seminal vesicle and cauda epididymis and normal serum follicle-stimulating hormone (3). The hallmarks of CBAVD include azoospermia, reduced semen volume (<1 ml), pH value (≤7.0) and seminal fructose, as well as decreased production of spermatozoa in the testicles (4).

CBAVD may occur as an isolated manifestation or as an atypical symptom of cystic fibrosis (CF), one of the most common autosomal recessive genetic disorders in the Caucasian population (5). CF has an incidence of ~1:2,500 and is usually caused by mutations in the CF transmembrane conductance regulator (CFTR) gene (5). Since almost 97% of male patients with CF are infertile due to CBAVD with resulting obstructive azoospermia, it may be hypothesized that isolated CBAVD and CF have a common genetic origin (1). The anatomical defects associated with CBAVD arise at the embryonic stage, and normal development of the male reproductive tract may require CFTR or CFTR-mediated anion secretion (3,6). In addition, 60-70% of patients with CBAVD carry pathogenic CFTR mutations (Online Mendelian Inheritance in Man no. 602421), usually one severe and one mild, in compound heterozygosity (7,8). However, the exact role of CFTR in male reproductive physiology remains to be fully elucidated. The present review summarized recent findings linking CFTR gene mutations to CBAVD and highlighted the assisted reproductive techniques (ART) and genetic counseling provided clear diagnosis and treatment options for such patients. Literature databases, such as Pubmed (pubmed.ncbi.nlm.nih.gov/) and Medline (de.medline.eu/), and Shanxi Medical University library (library.sxmu.edu.cn/) were searched for articles on CFTR and male fertility with relevant keywords such as cystic fibrosis transmembrane conductance regulator gene (CFTR), Congenital bilateral absence of the vas deferens (CBAVD), variation, pre-implantation genetic diagnosis (PGD), and assisted reproductive techniques (ART).

2. Overview of CFTR structure and function

The CFTR gene was first cloned and identified by Riordan *et al* (9) in 1989. In humans, it is located on chromosome 7q31.2 and spans a total length of 230 kb comprising 27 exons; the composition of the gene is outlined in Table I (10). CFTR encodes a protein containing 1,480 amino acid residues, with a molecular weight of 168,138 Da (11). The CFTR protein is an ATP-bound glycosylated transmembrane protein located in the apical membrane of several types of exocrine epithelial cells, which provides a selective channel for chloride ions across the epithelium (12). The structure of CFTR consists of five domains, including two membrane-spanning domains (MSD-1 and -2) together forming a selective channel, two nucleotide-binding domains (NBD-1 and -2) and a regulatory domain (R domain) (Fig. 1). Each MSD is composed of six transmembrane helices, of whichTM1-6 constitute MSD-1 and TM7-12 constitute MSD-2, followed by a cytoplasmic NBD (13). The MSD-2 and NBD-1 modules are connected by the R domain, a regulatory region with disordered structure that is unique to the CFTR gene and not found in other transporters (14). The two MSD domains form a selective chloride channel and the two NBDs regulate the gating of this channel (15). Furthermore, phosphorylation of the R domain controls channel activity (15). The R domain contains several phosphorylation sites for protein kinase A, a cyclic AMP-dependent kinase that is highly conserved between species (16).

CFTR is expressed in epithelial tissues of various organs, including the pancreas, intestine, sweat glands and vas deferens, and participates in the secretion of substances and fluids and maintains the balance of electrolyte and homeostasis in the lumen (17). In the steady state, the chloride channel is blocked by anions or negative charges in the regulatory R domain. Phosphorylation of the R domain is required for channel activity (18). As the concentration of intracellular chloride ion increases, Protein Kinase A (PKA) is activated, causing the channel to open (19). Similarly, the extracellular chloride concentration also regulates channel gating, and increased concentrations of extracellular chloride promote the opening of channels (20). Thus, CFTR facilitates two-way permeability of chloride ions (20). Previous studies have suggested that, in addition to regulating chloride ion transport through epithelial cells, CFTR is involved in the regulation of various ion transporters, including epithelial sodium channels, chloride/bicarbonate exchangers, sodium/proton exchangers and water channels (20-22). Therefore, CFTR-dependent physiological processes have a key role in maintaining homeostasis of pH, ions and water levels in the fluids of secretory epithelium (21).

3. The CFTR gene and development of the vas deferens

The majority of male patients with CF and CBAVD have primary infertility (22), suggesting that the CFTR protein has an important role in the development of the male reproductive tract (23). CFTR mutations among patients with CBAVD (58.1%) are common but not as frequent as in patients with unilateral absence of vas deferens (75%), and a normal amount of functional CFTR protein may be required to ensure the normal development of the vas deferens (24). Mutations in the CFTR gene impair the normal function of chloride channels and prevent them from regulating the flow of chloride ions and water across the cell membrane, resulting in the production of highly viscous mucus by epithelial cells of the male genital tract (25). This mucus, in turn, causes blockage of the vas deferens during embryonic development, causing it to denature or even deteriorate.

In 12-18 week-old abortive fetuses with CFTR gene mutations, the vas deferens is obstructed and denatured by mucus, and the vas deferens may be further aggravated in embryonic development (26). Furthermore, Gaillard *et al* (26) demonstrated that the expression of the CFTR in epididymal epithelium in human fetuses carrying CFTR mutations at 10-33 weeks of gestation was low, resulting in the production of highly viscous mucus by epithelial cells of the male genital tract. In addition, CFTR mutations are absent in cases of CBAVD with renal abnormalities, suggesting that the mesonephric duct has an important role in the development of the vas deferens (26). It has also been demonstrated that fluid secretion is necessary for the normal development of the mesonephric duct (25). Abnormal fluid secretion impairs normal development, leading to hypoplasia of the mesonephric duct and degeneration during early embryonic development (25,26). Therefore, CFTR may have an important role in the development of the vas deferens and mutations of CFTR may be associated with the occurrence of CBAVD.

4. The CFTR gene in patients with CBAVD

Numerous studies have investigated the genetic link between CFTR mutations and the risk of CBAVD (27-30). A large number of CFTR mutant alleles have been found in patients with CBAVD. CFTR mutations display high heterogeneity in terms of spectrum and frequency (27,28). A total of 50,022 CFTR gene variants have been identified to date (National Center for Biotechnology Information; ncbi.nlm.nih.gov/variation/view/?q=1080%5Bgeneid%5D&assm=GCF_000001405.25), most minor allele frequency (MAF) of which are <0.01%. Only 605 variants are >1% and 347 variants are >5%. A total of 1.68% of CFTR gene variants are considered to be potentially pathogenic, while 1.16% are not known to cause functional changes and another 97.16% have not been characterized (27). In addition, 90.43% of the mutations involve sequences that are only a few bases long. Of these, missense mutations were the most common, accounting for 2.97% of all mutations, while gene rearrangement was rare, accounting for only 1.13% of mutations (27).

To date, >2,000 CF-causing CFTR mutations have been identified (Table II; genet.sickkids.on.ca/StatisticsPage.html). CFTR gene mutations are divided into six classes, according to the extent of CFTR protein downregulation (28-30). Class I mutations refer to the production of non-functional protein or non-functional mRNA products that are degraded by nonsense-mediated mRNA decay (31). Class I mutations usually result from splice site mutations, nonsense mutations, frameshift mutations or large fragment insertion-deletion, the most common ones being these three types (p.G542X, p.R533X and p.W1282X) of missense mutations (31). Class II mutations result in defects of protein maturity (32). A common

Table I. Description of the structure of the cystic fibrosis transmembrane conductance regulator gene.

A, Exons

Name	Historical name	Start, base position	End, base position	Length, basepairs
Exon 1	Exon 1	117,120,017	117,120,201	185
Exon 2	Exon 2	117,144,307	117,144,417	111
Exon 3	Exon 3	117,149,088	117,149,196	109
Exon 3	Exon 3	117,170,953	117,171,168	216
Exon 5	Exon 5	117,174,330	117,174,419	90
Exon 6	Exon 6a	117,175,302	117,175,465	164
Exon 7	Exon 6b	117,176,602	117,176,727	126
Exon 8	Exon 7	117,180,154	117,180,400	247
Exon 9	Exon 8	117,182,070	117,182,162	93
Exon 10	Exon 9	117,188,695	117,188,877	183
Exon 11	Exon 10	117,199,518	117,199,709	192
Exon 12	Exon 11	117,227,793	117,227,887	95
Exon 13	Exon 12	117,230,407	117,230,493	87
Exon 14	Exon 13	117,231,988	117,232,711	724
Exon 15	Exon 14a	117,234,984	117,235,112	129
Exon 16	Exon 14b	117,242,880	117,242,917	38
Exon 17	Exon 15	117,243,586	117,243,836	251
Exon 18	Exon 16	117,246,728	117,246,807	80
Exon 19	Exon 17a	117,250,573	117,250,723	151
Exon 20	Exon 17b	117,251,635	117,251,862	228
Exon 21	Exon 18	117,254,667	117,254,767	101
Exon 22	Exon 19	117,267,576	117,267,824	249
Exon 23	Exon 20	117,282,492	117,282,647	156
Exon 24	Exon 21	117,292,896	117,292,985	90
Exon 25	Exon 22	117,304,742	117,304,914	173
Exon 26	Exon 23	117,305,513	117,305,618	106
Exon 27	Exon 24	117,306,962	117,308,715	1,754

B, Introns

Name	Historical name	Start	End	Length, basepairs
Intron 1-2	-	117,120,202	117,144,306	24,105
Intron 2-3	-	117,144,418	117,149,087	4,670
Intron 3-4	-	117,149,197	117,170,952	21,756
Intron 4-5	-	117,171,169	117,174,329	3,161
Intron 5-6	-	117,174,420	117,175,301	882
Intron 6-7	-	117,175,466	117,176,601	1,136
Intron 7-8	-	117,176,728	117,180,153	3,426
Intron 8-9	-	117,180,401	117,182,069	1,669
Intron 9-10	-	117,182,163	117,188,694	6,532
Intron 10-11	-	117,188,878	117,199,517	1,064
Intron 11-12	-	117,199,710	117,227,792	28,083
Intron 12-13	-	117,227,888	117,230,406	2,519
Intron 13-14	-	117,230,494	117,231,987	1,494
Intron 14-15	-	117,232,712	117,234,983	2,272
Intron 15-16	-	117,235,113	117,242,879	7,767
Intron 16-17	-	117,242,918	117,243,585	668
Intron 17-18	-	117,243,837	117,246,727	2,891
Intron 18-19	-	117,246,808	117,250,572	3,765
Intron 19-20	-	117,250,724	117,251,634	911

Table I. Continued.

B, Introns				
Name	Historical name	Start	End	Length, basepairs
Intron 20-21	-	117,251,863	117,254,666	2,804
Intron 21-22	-	117,254,768	117,267,575	12,808
Intron 22-23	-	117,267,825	117,282,491	14,667
Intron 23-24	-	117,282,648	117,292,895	10,248
Intron 24-25	-	117,292,986	117,304,741	11,756
Intron 25-26	-	117,304,915	117,305,512	598
Intron 26-27	-	117,305,619	117,306,961	1,343

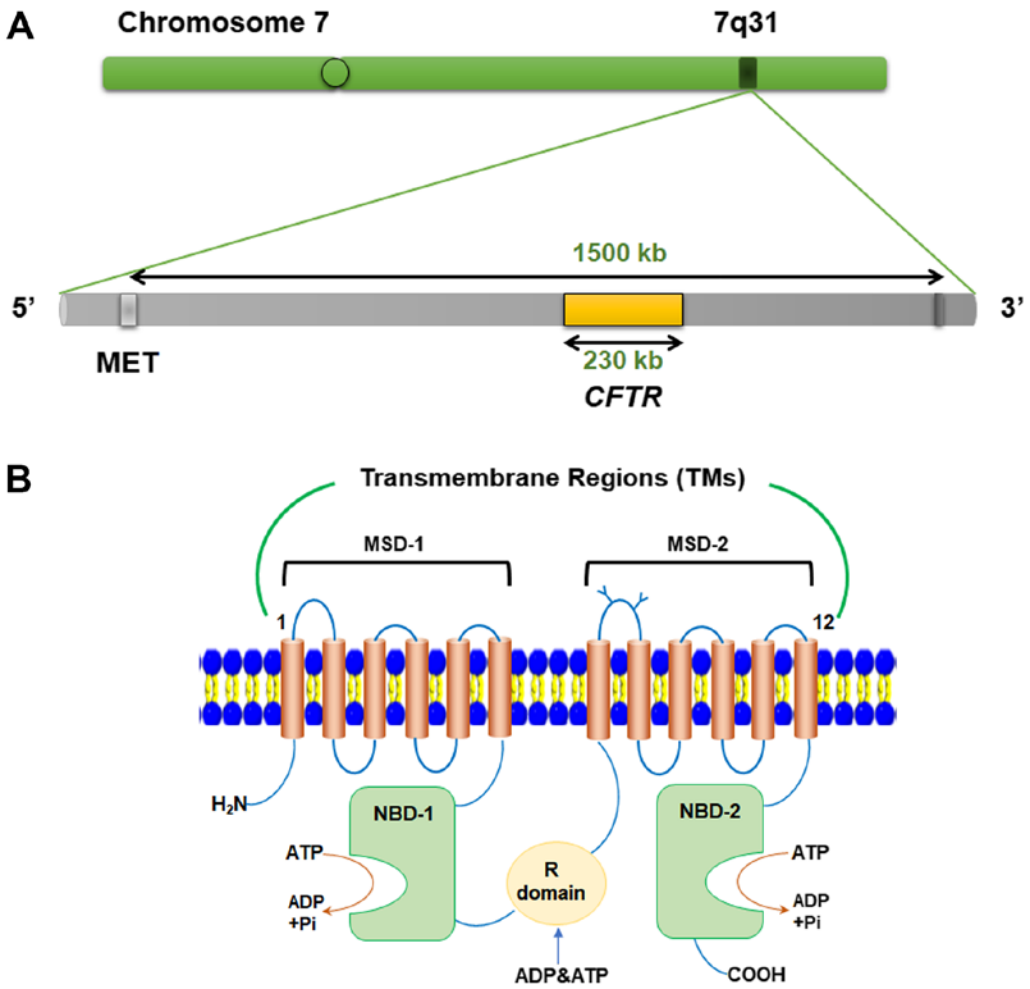

Figure 1. Schematic representation of the CFTR gene and protein. (A) Chromosomal localization of the CFTR gene. (B) CFTR protein structure and functional domain composition. CFTR, cystic fibrosis transmembrane conductance regulator; kb, kilobase; Met, methionine; TM, transmembrane region; MSD, membrane-spanning domain; NBD, nucleotide-binding domain; R domain, regulatory domain; Pi, pyrophosphate; H_2N, N-terminus; COOH, C-terminus.

example is the p.F508del mutation, which leads to abnormal folding of the CFTR protein, resulting in partial function and low CFTR protein concentration (32). Class III mutations, such as p.G551D, refer to defects in chloride channel gating function despite normal protein structure, which are usually caused by impaired binding of the NBD domain with ATP and hydrolysis (33). Class IV mutations in the CFTR gene, such as p.R117, p.HR334W and p.R347P, lead to abnormal regulation of chloride channel function, usually due to abnormal structure of MSDs (34). With class V mutations, protein function is normal, but CFTR protein synthesis is reduced (35). For instance, the c.3717+12191 C>T mutation in intron 19 causes leakage in transcription of exon 19, resulting in a decrease in mRNA to only 5-10% of the normal mRNA levels (35).

A novel class of mutations, Class VI, has recently been identified, which may be further divided into subclasses A

Table II. Mutations in the cystic fibrosis transmembrane conductance regulator.

Mutation type	Number of known mutations	Frequency
Missense	810	38.87
Frameshift	336	16.12
Splicing	229	10.99
Nonsense	175	8.40
In-frame indel	43	2.06
Large indel	59	2.83
Promoter	17	0.82
Sequence variation	269	12.91
Unknown	146	7.01

Mutation frequency refers to the frequency at which mutations in the CFTR detected make up this locus in all of the DNA. Indel, insertion-deletion.

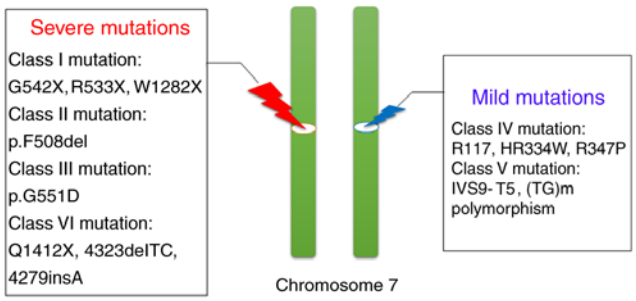

Figure 2. Phenotypic variability in patients with congenital bilateral absence of the vas deferens according to the type of CFTR mutation. Class I mutations refer to the production of non-functional protein or mRNA products that are degraded by nonsense-mediated mRNA decay. Class II mutations result in defects of protein maturity. Class III mutations refer to defects in chloride channel gating function despite normal protein structure. Class IV mutations lead to abnormal regulation of chloride channel function. Class V mutations cause normal protein function but CFTR protein synthesis is decreased. Class VI-A mutations lead to instability of CFTR protein, with a half-life reduced to only about one-fifth of normal proteins. Class VI-A mutations usually affect interactions between ion channels. Red represents severe mutations; green represents mild mutations. CFTR, cystic fibrosis transmembrane conductance regulator; del, deletion; ins, insertion; IVS, intervening sequence.

and B (36). Class VI-A mutations lead to instability of CFTR protein, with a half-life reduced to only about one-fifth of normal proteins. Examples of Class VI-A mutation include p.Q1412X, c.4323delTC and c.4279insA, which result in the loss of 70-100 bp of mRNA and abnormal folding of the C-terminal end of the CFTR protein (36,37). Class VI-B mutations, such as the p.G551D missense mutation, usually affect the interaction between MSDs of the CFTR protein (38).

Class I, II and VI mutations are generally considered severe, with near-complete loss of gene function. The transcription level of the CFTR gene is <3%, which is easy to cause CF and high clinical mortality (31,32,36). By contrast, class IV and class V mutations are mild mutations with slightly higher residual function, accounting for 3-10% of all CFTR mutations, and lead to CF-associated diseases, such as p.R117H-T7, lead to CBAVD (36).

A meta-analysis by Yu *et al* (8) suggested that 78% of patients with CBAVD had at least one type of CFTR gene mutation, among which 48% had two simultaneous mutations. Most of these mutations in patients with CBAVD were point mutations (class IV or V) with a mild phenotype, with few (<1%) gene deletions or rearrangements. In addition, 15-25% of patients with CF displayed gene rearrangements (Fig. 2). The mutation frequency and genomic region of CFTR in which mutations occur vary by region and ethnicity. For instance, the CFTR mutation rate in Caucasian populations is significantly higher than in Asians. Furthermore, CF is the most common lethal autosomal recessive genetic disease in Caucasians (8,39). The most common mutant alleles in Caucasians with CBAVD are p.F508del, c.IVS9-10 T5 and p.R117H, with frequencies of 13-21, 22-29 and 2-4%, respectively (8,39).

A severe heterozygous mutation on one allele combined with a heterozygous mutation on the other (88%), or two mild heterozygous mutations (12%) usually lead to the occurrence of CBAVD. However, a severe homozygous mutation (88%) or two severe heterozygous mutations (12%) may lead to the occurrence of CF (40). This suggests that CFTR gene mutations are ethnically specific and their non-coding regions may lead to mutations in CBAVD disease, while these types of mutations do not lead to CF.

5. Hotspot mutation types in patients with CBAVD

M470V. The p.M470V mutation is an amino acid substitution caused by A to G polymorphism of the nucleotide at position 1,540 in exon 11 of the CFTR gene. It is a missense mutation and affects the chloride channel activity of CFTR (41). Residue 470 codes for part of the first NBD domain, and both M470 and V470 may lead to the synthesis of a completely glycosylated CFTR protein. However, the chloride channel activity of the M470 variant is twice as high as that of V470 (41,42). Residue 470 also interacts with other sites on the CFTR gene, thereby influencing the expression levels of the CFTR protein (43). The M470V mutation is frequently combined with the (TGm) and Tn polymorphisms. The expression of the V470 CFTR variant decreases with increasing numbers of TG repeats and shorter T repeats, leading to an increased risk of CBAVD (43).

The ratio of M470 to V470 varies among different populations. This ratio is normally ~1:1 in the healthy Caucasian population. However, the ratio in the normal and infertile population remains to be determined. Furthermore, in Chinese patients with CBAVD, the ratio of M470 to V470 is 0.7:1, in line with the Hardy-Weinberg equilibrium, and it is thought to be unrelated to the pathogenesis of CBAVD (44).

p.F508del. p.F508del refers to the deletion of three bases (CTT) at positions 1,653-1,655 in exon 11. The resulting deletion of residue F508 of the CFTR protein leads to abnormal protein folding and a significant reduction in CFTR protein levels on the cell membrane of exocrine gland epithelial cells. Failure to form effective chloride channels on the epithelium, in turn, results in severe impairment of small molecule transport (45). p.F508del is the most common mutation in Caucasian patients with CBAVD, with a mutation frequency ranging from 35 to 74.6% (45). However, the incidence of this mutation is low in Asians (46,47).

p.D1152H. p.D1152H mutation is an amino acid substitution caused by G to C polymorphism at position 3,586 in exon 21 of the CFTR gene. This mutation is a class IV mild mutation, resulting in partial CFTR protein function and mild clinical manifestations of CF disease. In males, the major clinical manifestation is CBAVD (48). The incidence of the p.D1152H mutation is 12% in Ashkenazi Jewish (49) and 6.27% in Hispanic and African American individuals (50), which included pre-marital and pre-natal screening in certain areas with a high incidence of CF (48).

p.R117H. p.R117H results from a G to A substitution at position 4,482 of exon 4; the resulting change from arginine to histidine is considered a mild mutation. However, pR117H combined with the intervening sequence (IVS) 9-10 T5 variant leads to severe clinical manifestations of CF (51). In addition, p.R117H combined with IVS9-10 T9 does not cause disease. Furthermore, when combined with IVS9-10 T7, this mutation frequently leads to CBAVD and results in CF when combined with IVS9-10 T5 (52). p.R117H has a high incidence (~3%) in Caucasian patients with CF.

Mutations in the promoter region. Promoters regulate gene transcription levels by interacting with *trans*-acting factors (17,53). The CFTR gene promoter is a housekeeping-type, GC-rich (<65% GC content) promoter lacking the typical TATA box, and its specific *cis*-acting element location and regulatory mechanism are poorly characterized (53). Yoshimura *et al* (54) suggested that the core sequence of the CFTR gene promoter may be located ~3.8 kb upstream of the ATG transcription initiation site and contains conserved sequences acting as binding sites for several important transcription proteins, such as CTCC-binding factor (CTCF), activator protein-1 (AP-1), specificity protein 1 (SP1), greater curvature (GRE), carbapenem-resistant (CRE), CCAAT/enhancer-binding protein (C/EBP) and Y-box proteins (55). Giordano *et al* (56) examined the CFTR gene promoter in patients with CF and identified 23 mutations. All of these were point mutations that affected transcription, resulting in lack of regulation, upregulation and downregulation of the CFTR gene (56). Lopez *et al* (67) demonstrated that the forkhead box I1 (FOXI1) transcription factor was able to downregulate CFTR gene transcription. Indeed, the c.-165G>A promoter mutation enhanced FOXI1 binding, thereby inhibiting transcription of the CFTR gene. Feng *et al* (53) recently demonstrated that the c.-195C>A promoter mutation significantly enhanced the inhibitory effect of specificity protein (SP) on CFTR expression.

Intron 9-10 (TG)m and Tn polymorphism. (TG)m and Tn polymorphism inIVS 9-10 of the CFTR gene are associated with the occurrence of CBAVD. Tn refers to the length of a noncoding thymidine sequence. The Tn sequence is usually 7 or 9 bases long (T7 and T9, respectively) in the normal population, while the incidence of 5T is 5.2% (57,58). The shorter the Tn sequence, the lower the transcription level of the CFTR gene.

In patients who are homozygous for the IVS9-10 5T (c.1210-7_1210-6delTT variant, formerly known as IVS8 5T), a sequence of 5 consecutive thymidines in intron 9 may cause alternative splicing of exon 10 mRNA, resulting in missing transcription of the CFTR gene. This, in turn, causes a reduction in the synthesis of CFTR protein with normal functions (only 10% of the normal protein); these patients with CBAVD do not exhibit other symptoms of CF (Fig. 3A) (30). Exon 10 encodes 60 amino acids of the CFTR protein NBD-1. Thus, a mutant CFTR protein lacking this amino acid sequence loses chloride channel activity, thus reducing the permeability of chloride to the epithelial cell membrane of the exocrine gland and increasing the reabsorption of sodium ions in the exocrine fluid, eventually leading to CF and associated diseases including chronic obstructive pulmonary disease, pancreatic exocrine dysfunction and meconium ileus (59).

Radpour *et al* (60) examined the distribution of Tn alleles in 106 patients with CBAVD and demonstrated that the incidence of T5 was 25.94%, although T5 distribution was not investigated among males with normal fertility. However, the incidence of Tn polymorphism in patients with CBAVD varies with ethnicity, ranging from 13 (Asian) to 43.7% (Caucasian) (61). Disset *et al* (62) identified a rare IVS9 T3 mutation in one case. Functional experiments demonstrated that this mutation led to severe cystic fibrosis (62), consistent with the hypothesis that leakage in exon 10 transcription becomes more serious with the decrease in length of the Tn sequence (59). The T5 variant is currently considered to be a mild CFTR gene mutation rather than a polymorphism.

Furthermore, the (TG)m (c.1210-34) polymorphism also affects the transcription of exon 10 (Fig. 3B). In association with the T7 variant, loss of exon 10 transcription caused by TG11 and TG12 is 2.8 and 6.0 times more likely than with TG10, respectively (63). Thus, (TG)m and Tn synergistically affect the transcription of exon 10. Indeed, the greater the number of TG repeats, and the shorter the number of T repeats and the higher the probability of transcription loss of exon 10 is (63). IVS9-10 (TG)m Tn is a common mutation type of the CFTR gene. Furthermore, the frequency of this mutation in Chinese patients with CBAVD is slightly higher than that in Caucasian patients (44).

Intron 10-11 (TAAA)n short-tandem repeats. Short-tandem repeats are widely distributed in the genome and the length of repeats is closely associated with the occurrence of diseases, such as neuromuscular disorders (64) and gynecomastia (65). Previous studies have also demonstrated that short-tandem repeats in promoters and introns may affect gene transcription (64-66). A large number of dimer and tetramer short-tandem repeats have been identified in the noncoding region of the CFTR gene that affect its expression. (TAAA)n is a short-tandem repeat composed of four bases in IVS10-11 that may impair the transcription of exon 10. However, it does not affect the transcription of exon 10 as Tn in intron 9-10 does. Lopez *et al* (67) suggested that FOXI1 transcription factor binds to IVS10-11 (TAAA)n short-tandem repeats to negatively regulate the expression of the CFTR gene. With decreasing numbers of repeats, binding of FOXI1 transcription factor and promoter was enhanced, thus reducing the transcriptional levels of the CFTR gene. Furthermore, combination with the c.-165G>A mild promoter mutation or IVS9-10 (TG)mTn may lead to the occurrence of CBAVD (67).

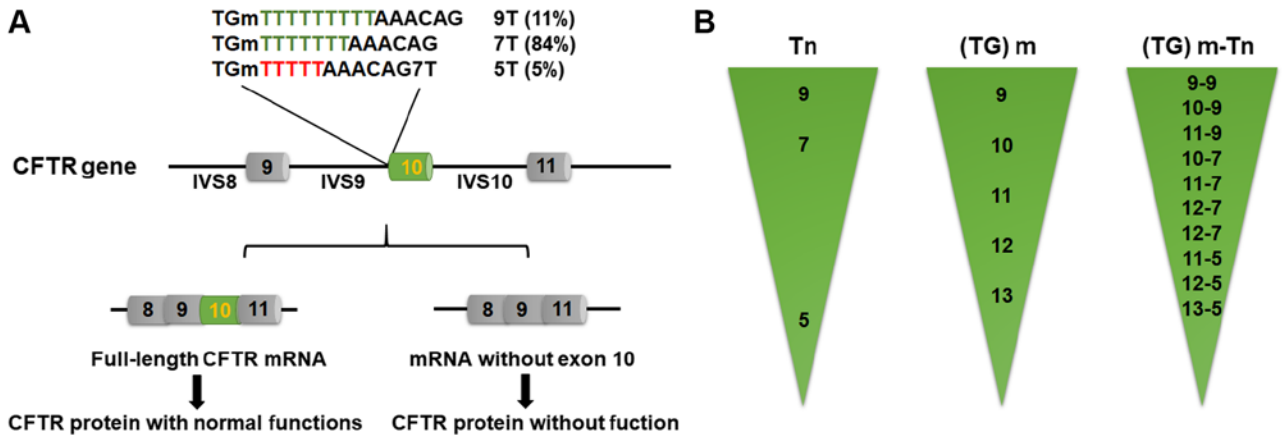

Figure 3. (TG)m and Tn polymorphism in IVS9-10 of the CFTR gene is associated with the occurrence of congenital bilateral absence of the vas deferens. (A) The T5 allele promotes CFTR exon 9 skipping in humans. (B) Effects of different (TG)m or Tn polymorphism sites or haplotypes on the amount of CFTR chloride channel activity. Green triangles represent the amount of functional CFTR, decreasing amounts of functional CFTR are obtained from top to bottom. CFTR, cystic fibrosis transmembrane conductance regulator; IVS, intervening sequence.

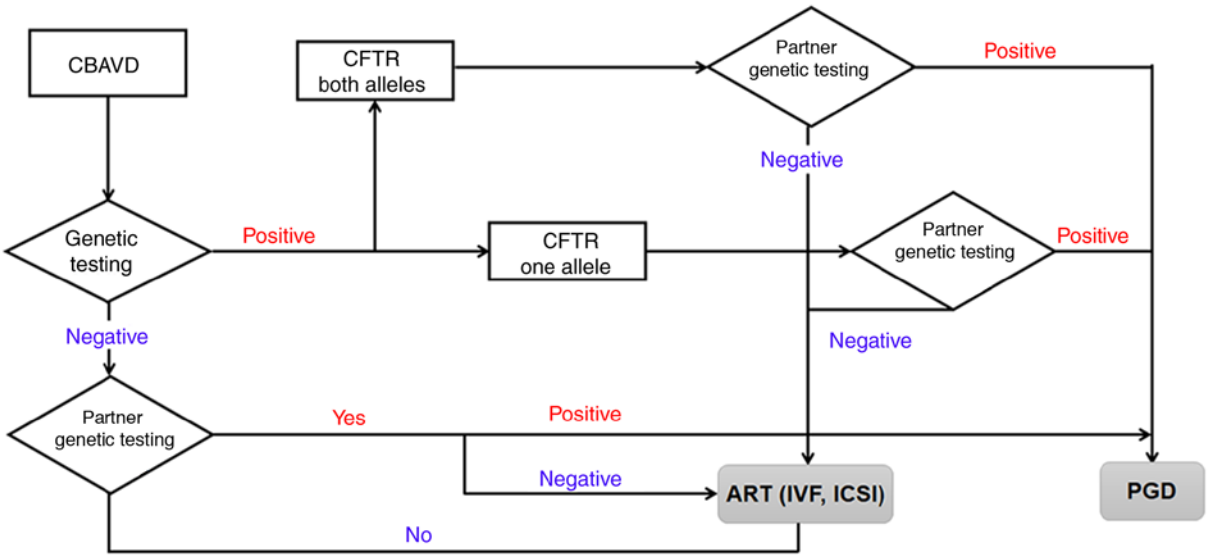

Figure 4. Systematic genotyping strategy for couples with CBAVD. Genetic counseling should therefore be provided to CBAVD couples, and the male partner should be tested for CFTR mutations in order to determine the risk of having a CBAVD child. In addition, for all couples with positive results, PGD and PND are recommended. CBAVD, congenital bilateral absence of the vas deferens; CTFR, cystic fibrosis transmembrane conductance regulator; ART, assisted reproductive techniques; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; PGD, pre-implantation genetic diagnosis; PND, pre-natal diagnosis.

Although the CFTR gene was identified >20 years ago, the relationship between its genotype and clinical phenotype remains controversial, mostly due to the wide variety of CFTR gene mutations and differences across ethnicities. Furthermore, other factors may also be involved, including environmental factors, viruses or bacteria (27,50,61).

In addition, it has been observed that 6-15% of patients with CBAVD did not carry any CFTR gene mutations (1), further highlighting possible limits of detection during diagnosis or the possibility that other genetic mutations are present in certain patients.

6. Assisted reproductive techniques and genetic counselling

Compared with patients with CF, patients CBAVD are more likely to have mild CFTR gene mutations (class IV and V) associated with CFTR protein dysfunction. Most patients with CBAVD have normal spermatogenic function, and their spouses may be impregnated via testicular tissue sperm extraction (TESE) or intracytoplasmic sperm injection (ICSI), a procedure, through which a single sperm is injected into the cytoplasm of a mature oocyte to obtain a viable embryo (68). The first pregnancy for a couple in whom the male had CBAVD was reported in 1987 (68). A recent meta-analysis suggested that the results of ICSI were not affected by the source of sperm (fresh, frozen, epididymal or testicular), but a lower rate of fertilization and a higher rate of miscarriage were observed in patients with CBAVD as compared with those with acquired aspermia (69).

Due to possible inheritance of the CFTR mutation and risk of CF occurrence, genetic counseling prior to the use of assisted reproductive technology (ART) is particularly important,

especially for patients with a CBAVD familial history. To date, CFTR gene mutation detection has been applied in pre-implantation genetic diagnosis (PGD) in the USA and certain European countries such as the Netherlands (70), Russia (71) and France (72). CFTR mutation screening as part of a PGD screening program may select embryos carrying a healthy CFTR gene, which has certain clinical significance for couples affected by CBAVD and their potential children. Liu *et al* (70) reported the first successful PGD in a couple affected by CBAVD, in which both partners were heterozygotes with p.F508del mutations. In this instance, three carrier embryos were implanted and a healthy male infant was born. The presence of CF or CBAVD-associated mutations in the male partner does not currently appear to significantly affect *in vitro* oocyte fertilization, embryo implantation rates or successful delivery of asymptomatic offspring after PGD (73,74).

For a couple affected by CBAVD with CFTR deficiency planning to have their own children, the risk for both male and female offspring to have CF or associated diseases, and for male offspring to have CBAVD depends on whether the female partner is a carrier, as one mutant allele is always inherited from the male. If the female does not carry the CFTR gene mutation, the probability of CBAVD in the male offspring is low, estimated to be <1%. The inheritance of CBAVD is more complex than that of CF, as genetic analysis may include but not exclude the diagnosis of reproductive CF. Furthermore, when rare mutations are detected in males or females, the risk of CF or CBAVD in the offspring may be unpredictable. The couple should be informed that tests cannot detect all mutations in the gene; therefore, negative mutation screening may reduce but not eliminate the risk of being a carrier.

Clinical examination and follow-up of children born to couples affected by CBAVD are of great significance, as it may provide information regarding the variability in phenotypes associated with CFTR mutations. Identifying a CFTR mutation in a male affected by CBAVD is important not only for the patient himself, but also for his family. Healthy siblings of a male child born to a parent with CBAVD have a 50% chance of being carriers of CF. Therefore, both the patient and their partner should be screened for CFTR gene mutations. If no mutation is detected in the male partner, it is not certain that the unborn child will be free of CF, and the risk may be <1:1,000 (27). Among male infertile heterosexual couples not affected by CF, the risk of CF in the offspring was no different from that of couples in the general population. The risk of infertility in their male offspring remains elusive. In addition, for all couples with positive results, PGD and prenatal diagnosis (PND) are recommended, together with genetic counseling (Fig. 4).

7. Conclusions

CBAVD is an atypical manifestation of CF caused by mutations in the CFTR gene, which frequently leads to male infertility. The present review summarized recent findings linking the CFTR gene to male fertility and highlighted the progress made in understanding the role of CFTR in reproductive events pertinent to male fertility. Genetic counseling for couples affected by CBAVD remains challenging. Characterizing the mutation spectrum of the CFTR gene and its association with the pathogenesis of CBAVD may prove conducive to disease diagnosis and genetic risk assessment prior to IVF, thereby reducing the incidence of this disease or the rate of CFTR mutations in infants resulting from ART. Consistent guidelines are also required concerning the extent of mutational analysis for the CFTR gene during the diagnosis of CBAVD, as well as options available for couples affected by CBAVD in association with PND and PGD.

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Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the present study.

Authors' contributions

XC and XJ designed the study. XW and QL interpreted the data. All authors wrote, read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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