Genetics of *CFTR* and male infertility

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Abstract: Cystic fibrosis (CF) is a rare autosomal-recessive disorder manifested as multisystem organ dysfunction. The cystic fibrosis transmembrane conductance regulator (CFTR) protein functions as an ion transporter on the epithelium of exocrine glands, regulating secretion viscosity. The *CFTR* gene, encoded on chromosome 7, is required for the production and trafficking of the intact and functional CFTR protein. Literally thousands of human *CFTR* allelic mutations have been identified, each with varying impact on protein quality and quantity. As a result, individuals harboring *CFTR* mutations present with a spectrum of symptoms ranging from CF to normal phenotypes. Those with loss of function but without full CF may present with CFTR-related disorders (CFTR-RDs) including male infertility, sinusitis, pancreatitis, atypical asthma and bronchitis. Studies have demonstrated associations between higher rates of *CFTR* mutations and oligospermia, epididymal obstruction, congenital bilateral absence of the vas deferens (CBAVD), and idiopathic ejaculatory duct obstruction (EDO). Genetic variants are detected in over three-quarters of men with CBAVD, the reproductive abnormality most classically associated with *CFTR* aberrations. Likewise, nearly all men with clinical CF will have CBAVD. Current guidelines from multiple groups recommend *CFTR* screening in all men with clinical CF or CBAVD though a consensus on the minimum number of variants for which to test is lacking. *CFTR* testing is not recommended as routine screening for men with other categories of infertility. While available *CFTR* panels include 30 to 96 of the most common variants, complete gene sequencing should be considered if there is a high index of suspicion in a high-risk couple (e.g., partner is *CFTR* mutation carrier). CF treatments to date have largely targeted end-organ complications. Novel CFTR-modulator treatments aim to directly target CFTR protein dysfunction, effectively circumventing downstream complications, and possibly preventing symptoms like vasal atresia at a young age. Future gene therapies may also hold promise in preventing or reversing genetic changes that lead to CF and CFTR-RD.

Keywords: Cystic fibrosis transmembrane conductance regulator protein (CFTR protein); congenital absence of vas deferens; cystic fibrosis (CF); *CFTR*

Introduction

The *CFTR* (cystic fibrosis transmembrane conductance regulator) gene, encoded on the long arm of chromosome 7 (7q31), consists of 27 coding exons spanning 230 kb (1).

The transcribed 6.5-kb mRNA is translated into a 1,480 amino acid protein, the sequence of which directly affects protein folding and is crucial to proper functioning. Genetic alterations in *CFTR* can affect the final protein product in

Table 1 *CFTR* mutation classes

Class	*CFTR* defect	Worldwide frequency	Common mutations	Phenotype severity
1	Protein synthesis	10% (Ashkenazi 50%)	G542X, W1282X	Severe
2	Protein processing	70%	F508del, N1303K	Severe
3	Gating	2–3%	G551D, R560T	Severe
4	Conduction	Uncertain (<2%)	R117H, R347P	Mild
5	Reduced quantity	Uncertain (<1%)	3349+10kbC>G, IVS8-5T	Mild
6	Reduced stability	Uncertain	Q1411X	Mild

a myriad of ways, from altered functionality to decreased production or combinations thereof (2).

Properly configured, CFTR acts as a cyclic AMP-activated ion channel, driving bicarbonate and chloride exchange and thus leading to water movement and decreased secretion viscosity in many organ systems (2,3). Secretion viscosity is central to the physiologic roles of the lungs, exocrine pancreas, gastrointestinal system, and male reproductive tract. Significant biallelic *CFTR* abnormalities result in cystic fibrosis (CF), a severe disorder comprising symptoms in several or all of the organs noted above. Impaired chloride reabsorption in sweat glands also leads to the classic "salty sweat" found in patients with CF.

Only certain *CFTR* mutations manifest as CF. Less severe mutations or variants can present with limited signs or symptoms. Of particular interest to male infertility specialists, men with CF or CFTR-related disorders (CFTR-RDs) may manifest a spectrum of sperm obstructive processes, ranging from decreased to absent sperm counts. The most classically cited male infertility CFTR-RD is congenital bilateral absence of the vas deferens (CBAVD).

These phenotypes will obviously affect reproductive treatment options but should also spur genetic evaluation and counseling. With *CFTR* mutation carrier rate up to 4% in some American populations, the threshold for genetic testing should be low when evaluating men with any reproductive tract abnormalities (4). This review will serve to describe *CFTR* abnormalities, variable phenotypic presentations, genetic diagnostic methods, and treatment options with specific focus on relationships to male infertility. Sperm retrieval and utilization in men with obstructive azoospermia (OA) secondary to *CFTR* mutations are discussed in another article in this issue.

Diseases associated with CFTR dysfunction

CFTR variants

The Cystic Fibrosis Mutation Database, maintained by The Hospital for Sick Children in Toronto, Canada, is charged with keeping an up-to-date listing of identified *CFTR* mutations (5). As of the writing of this manuscript, 2,074 mutations are currently detailed, each of varying prevalence. The nomenclature of each variant was updated in 2010 to the standards recommended by the Human Genome Variation Society. In order to retain relevance to the body of literature on the topic, this article will utilize conventional *CFTR* mutation designations.

Researchers have classified *CFTR* mutations into six different classes: protein synthesis (Class 1), protein processing (Class 2), gating (Class 3), conduction (Class 4), reduced quantity (Class 5), and reduced stability (Class 6) defects as detailed in *Table 1* (2,6,7). Each functional class may be caused by various genetic aberrations including base deletion, substitution, insertion, or complete gene duplication or deletion. The F508del mutation, for example, the most common CF abnormality, represents a protein processing (Class 2) mutation due to a three-nucleotide deletion, removing a crucial phenylalanine amino acid (6). Without it, CFTR protein cannot fold properly resulting in rapid degradation. While classically considered a Class 2 (protein processing) mutation, F508del also results in gating defects (Class 3) and reduced protein stability (Class 6) (7). *CFTR* mutations may more appropriately be considered part of a complex Venn diagram as discussed in more recent reviews.

Another common variant site is the polythymidine tract at the terminal end of intron 8. Repeat thymidine bases influence the splicing of exon 9 with inversely proportionate

splicing to the number of bases. Thus, 7T or 9T sequences generally result in normal exon 9 splicing while the shorter 5T variant (also known as IVS8-5T, a Class V mutation) results in frequent exon 9 skipping, leading to a reduced quantity of functional CFTR protein (8). This variant does not cause classic CF, but may increase the severity of CF among those with two *CFTR* gene mutations or reduce functional CFTR protein leading to CFTR-RDs. With an allele frequency of 5%, the carrier frequency of 5T variants is approximately 10% in the general population (9,10).

The Clinical and Functional TRanslation of *CFTR* project (CFTR2), supported by the US Cystic Fibrosis Foundation, details unique *CFTR* variants and the propensity of each for functional impairment (11). Variants are characterized as CF-causing, variants of varying clinical consequence, non-CF, and those of unknown significance. Homozygous expression of F508del is classically associated with a severe CF phenotype. Varying genotypic combinations of the variant categories noted above however lead to a greater spectrum of CF and CFTR-RD phenotype severity.

Phenotypes of CFTR dysfunction

Contrary to many genetic disorders, *CFTR* mutations are not reflected by an "all or none" disease state. The phenotypes of CF and CFTR-RDs, rather, are similar, but vary based on the number and severity of organ systems involved. Symptoms can range from multisystem organ failure with CF, through more mild CFTR-RDs, to no symptoms whatsoever due to differential tissue *CFTR* expressivity and sensitivity. Most CBAVD patients, even those with two identified *CFTR* variants, have no lung disease, indicating the relative sensitivity of the vasa to *CFTR* dysfunction. This variability is believed to be secondary to tissue-specific alternative splicing which occurs more in the vasa compared to other tissues (12). Likewise complicating the picture, *CFTR* variants have heterogeneous effects on protein levels and functionality. Phenotypic symptom severity can even vary among individuals with identical *CFTR* genotypes secondary to other gene modifiers (1).

The impairment in CFTR protein quantity or quality varies widely based on genetic mutation class and type. The combination of two *CFTR* alleles ultimately determines the presence or absence of symptoms and disease severity. Classically two pathogenic CF-causing mutations, such as F508del, lead to CF. The combination of one CF-causing and one non-CF-causing mutation, a compound heterozygote, often allows the *CFTR* gene to retain some level of functionality. Individuals with these permutations can overlap with the CF phenotype, leading to possible male infertility with a small percentage reporting mild CF symptoms and bringing into question possible subclinical effects (13). Pulmonary evaluation of eight men with isolated CBAVD and *CFTR* mutations by Gilljam *et al.* revealed higher rates of inflammatory markers and opportunistic bacteria compared to controls (14). Appropriate referrals should be made for men presenting with CFTR-related infertility and any abnormal pulmonary signs or symptoms.

Cystic fibrosis

CF, as noted, is caused by significant aberrations in CFTR protein function. As a result, epithelial secretions are pathologically viscous, resulting in multisystem disease affecting the respiratory tract, exocrine pancreas, gastrointestinal system, and male reproductive tract. Clinical symptoms include chronic cough, recurrent sinusitis, obstructive lung disease, recurrent pulmonary infections, and male infertility (1). Pulmonary disease remains the major cause of CF morbidity and mortality (15). With advanced care however, life expectancy has increased significantly. According to the 2017 CF Patient Registry Annual Data Report, the current median survival is 43.6 years compared to 32.7 years as recently as 2002 (16). Now that men with CF are living well into and beyond their reproductive years, infertility care is coming to the forefront but the challenge remains that essentially all have bilateral vas deferens absence.

CFTR-RDs

A subset of individuals may present with genetically related, or CFTR-RDs, without meeting full criteria for CF (17). The spectrum of phenotypic presentations of these individuals with varying *CFTR* mutations (often heterozygous) is reflective of the complex landscape of genotypic subtypes. Relevant to this review, men with *CFTR* abnormalities can have isolated infertility with a range of sperm transport obstructive processes as described in *Table 2*. The central theme is a higher relative frequency of severe compared to mild *CFTR* mutations in men with CF trending progressively downwards in men with CBAVD, congenital unilateral absence of the vas deferens (CUAVD), epididymal obstruction, and oligospermia.

The most classic reproductive tract abnormality

Table 2 Potential reproductive tract abnormalities in men with *CFTR* abnormalities

Reproductive tract abnormality	*CFTR* abnormality prevalence*	Severity of *CFTR* abnormality (allele 1; allele 2)
Oligospermia	17.5% (18)	Mild; wild
Epididymal obstruction	34–47% (9,19)	Severe/mild; wild
Ejaculatory duct obstruction and seminal vesicle abnormalities[†]	86% (20)	Severe/mild; mild/wild
CUAVD	46% (21)	Severe/mild; mild/wild
CBAVD	78% (22)	Severe/mild; mild/wild
CF with CBAVD	95% (23)	Severe; severe

*, one or more *CFTR* mutations; [†], EDO of idiopathic etiology; Wild denotes wild-type allele or no detectable mutation. CUAVD, congenital unilateral absence of the vas deferens; CBAVD, congenital bilateral absence of the vas deferens; EDO, ejaculatory duct obstruction.

associated with *CFTR* mutations, isolated CBAVD, is often initially detected at the time of urology evaluation for male infertility. Clinical exam typically reveals complete absence of bilateral vas deferens and distal epididymis with seminal vesicle hypoplasia or absence (24). CBAVD represents the only male infertility-causing genetic mutation that can be detected on physical examination. Laboratory testing will demonstrate OA with normal gonadotropins and reproductive hormones. Comprehensive semen analysis will typically reveal no sperm with low ejaculate volume (<1 mL), low pH (<7), and reduced fructose level (25).

Bilateral vasal absence is highly suggestive of *CFTR* allelic abnormalities. In a 2012 meta-analysis by Yu *et al.*, 78% of men with CBAVD were noted to have at least one identified *CFTR* mutation (22). Delving deeper, 46% of men in the included studies had biallelic mutations and 28% had a single mutation. A criticism of the review is that many of the individual studies were conducted in earlier years when fewer *CFTR* mutations were known and detectable (26). Among those men with heterozygous mutations, the most common pairing was F508del/IVS8-5T, comprising 17% of CBAVD cases. All other combinations were found in less than 3% of cases. While conventional thinking suggests that CBAVD is caused by biallelic variants, the data above which includes studies with exhaustive *CFTR* screening methods (that theoretically would capture any *CFTR* variant) clearly demonstrates a subset of CBAVD men with a wild type allele, obligatorily *trans* to a *CFTR* variant (9). In these men, there are clearly other genetic regions or modifiers reducing CFTR protein quantity or quality and resulting in vasal atresia (27).

As if not heterogeneous enough, some men with congenital absence of the vas deferens have completely normal *CFTR* genotypes (two wild-type alleles). In these cases, vasal absence is thought to be secondary to alternative genetic or toxic insults affecting Wolffian duct development. To review, the rudimentary embryologic Wolffian duct divides into two limbs near seven weeks of development. One limb forms the basis for ureteral and renal development while the other is responsible for much of the sperm transport system, namely the distal two-thirds of the epididymis, vas deferens, and seminal vesicle (28). Any insult to the Wolffian duct prior to seven weeks then results in abnormalities to the male reproductive structures in addition to the ipsilateral kidney (29). In contrast, renal development is typically normal in cases of CBAVD, suggesting that vasal atresia in men often harboring *CFTR* mutations occurs after the critical seven-week time point. A single center review of over a decade of men with CBAVD revealed only 17 of 168 (10.1%) men with unilateral renal agenesis (URA) (29). Schlegel *et al.* reported a similar 11% rate of URA in 84 men presenting with CBAVD (30). While these rates are low, the incidence of URA in CBAVD is certainly not negligible.

If a unilateral Wolffian duct is affected early during embryogenesis, specifically before seven weeks, vasal and renal development may be jointly affected leading to unilateral agenesis (29). In a meta-analysis reported in early 2019 by Cai *et al.*, URA was detected in 22% of 141 men with CUAVD (21). Contrast this to the 10% rate of URA in men with CBAVD noted above. Among men with CUAVD, 46% had one or more *CFTR* mutations (one detected in 27% and two in 5%). The most frequently abnormal alleles were IVS8-5T (9%) and F508del (4%). Interestingly, the aggregate frequency of F508del/IVS8-5T, the most common heterozygous genotype in CBAVD, was 0% in

men with CUAVD. It remains unknown why some *CFTR* mutations cause CUAVD while others lead to CBAVD. Future research may also help elucidate alternative genetic etiologies of CUAVD.

Given the association between vasal and renal development and maldevelopment, the current American Urological Association (AUA) Best Practice Statement on evaluation of azoospermic men recommends offering screening renal ultrasound to men with CUAVD or men with CBAVD and no identified *CFTR* abnormalities (31). The apparent subpopulation excluded from this recommendation is those with CBAVD and an identified *CFTR* variant. McCallum *et al.* reported a single center series including 17 men with URA and CBAVD, only three of which had a *CFTR* mutation (29). Cases of URA should be advised of solitary kidney precautions and annual follow up recommended for blood pressure, serum creatinine, and urinalysis monitoring.

Sperm obstructive processes at various levels and severity other than congenital vasal absence have additionally been linked to *CFTR* abnormalities. In 62 azoospermic men, Jarvi *et al.* detected *CFTR* variants in 20/25 (80%) men with CBAVD, 8/17 (47%) with OA, and only 2/18 (11%) with nonobstructive azoospermia (19). All men with OA were noted to have epididymal obstruction at the time of surgical sperm retrieval with palpable vasa. EDO, typically a result of prostatic surgery, infection, or cysts, has also been associated with *CFTR* mutations. In a small series of men with idiopathic bilateral EDO with abnormal seminal vesicles, 6/7 (86%) were found to have *CFTR* variants (20). There did not appear to be a connection between genetic variants and isolated seminal vesicle abnormalities in the same series.

Despite studies suggesting direct *CFTR* involvement in spermatogenesis, the prevalence of *CFTR* mutations among men with nonobstructive azoospermia (n=5/45, one F508del and four IVS8-5T) was not significantly different than that observed in the general population (32). Oligospermia, or reduced sperm number, on the other hand was noted in 14/80 (17.5%) men, a higher than expected frequency compared to the local population (18). While some have used this data to support the hypothesis that *CFTR* impacts spermatogenesis, it may more accurately reflect partial obstruction, another aspect of the *CFTR* phenotypic spectrum. The data supporting broad associations between *CFTR* mutations and various male reproductive tract abnormalities would suggest a low threshold for genetic testing.

Prevalence of CFTR variants and associated diseases

CF is an autosomal recessive disorder requiring maternal and paternal transmission of pathogenic *CFTR* mutations. Just as *CFTR* gene mutations vary by ethnic and racial background, so does CF prevalence. Incidence of CF is as high as 1 in 1,600–2,000 individuals of northern European descent (24,33). As such, CF represents the most common Caucasian life-limiting genetic defect (1). The reported rate of CF in other groups is much lower, affecting 1:15,000 African Americans and 1:31,000 Asian Americans (34). Overall, it is estimated that 30,000 individuals are currently living with CF in the United States.

The F508del mutation is the most common pathogenic allele identified in CF patients worldwide, detected in 66% of cases (35). Similar to the variability in CF disease, *CFTR* genotypic variants differ widely based on ethnicity and geographic location. Among individuals with CF, F508del is detected in 100% of Faroe Islanders, 85% of North Americans, 70–80% of northern Europeans, 50% of southern Europeans, 48% of African Americans, 46% of US Hispanics, 30% of Ashkenazi Jews, and rarely in American Indians (16,36,37). An alternative mutation, W1282X, surpasses F508del as the most common mutation in Ashkenazi Jews with CF.

As the frequency of clinical CF is relatively high so far as genetic disorders go, the carrier frequency is expectedly high, as high as 1 in 28 Americans of Northern European descent (4). Carrier frequency varies again by ethnic and racial background, being detected in 1 in 29 Ashkenazi Jews, 1 in 61 African Americans, and 1 in 118 Asian Americans (4,38,39). As such, the ethnicity of partners of men with CFTR-related infertility must be considered when predicting risk of mutations in future offspring.

CFTR variants have been clearly linked to obstructive male infertility processes as noted above (9,18-22). CBAVD is present in 95–98% of men with CF (40). Various reports cite the incidence of CBAVD as high as 1–2% of infertile men (1,41). CUAVD, which is less frequently associated with *CFTR* abnormalities, is estimated to occur in less than 1% of the population (42). A disconnect appears when compiling *CFTR* variant and congenital absence of the vas deferens data. Despite a *CFTR* carrier frequency among North American Caucasians of 4–5%, CBAVD is diagnosed in only 1–2% of infertile men and certainly less than 1% of the general male population (1,41,43). More simply put, most men with *CFTR* mutations have normal bilateral vasa.

Fitting these facts together suggests a genetic "dosing level" required for normal Wolffian duct development, below which absence of the vas deferens occurs.

Diagnostic approach

Initial evaluation of any infertile male begins with a history and physical examination followed by laboratory testing including comprehensive semen analysis and serum hormones (44). Often a semen analysis will be done before men are referred to a male infertility specialist. When low volume azoospermia is found, careful attention must be paid to the presence or absence of bilateral vasa and epididymides on exam. Even if CBAVD is detected, it is important not to ignore the other components of male testing, namely serum hormones, as up to 12% of men with CBAVD have been shown to have impaired spermatogenesis in addition to their obstructive process (45).

Genetic and functional testing form the gold standard for diagnosing CF and CFTR-RDs. CF is defined by one or more characteristic clinical features and evidence of abnormality in CFTR function such as biallelic pathogenic *CFTR* mutations, two abnormal quantitative sweat chloride tests, or transepithelial nasal potential difference characteristic of CF (1). Diagnosis can be confirmed in infants with elevated trypsinogen on newborn screening and findings of biallelic *CFTR* mutations or elevated sweat chloride test. Individuals presenting with findings or symptoms suggestive of a CFTR-RD should also undergo *CFTR* testing.

Multiple society guidelines, including those from the World Health Organization, American Society of Reproductive Medicine, and AUA, recommend *CFTR* mutation analysis in all men with CF or CBAVD (31,33,46). When reviewed, the literature contains a high strength of evidence to support these statements (33). Despite the higher prevalence of *CFTR* mutations and variants in men with epididymal obstruction and oligospermia, routine mutation analysis has not been recommended for these categories of infertility to date. The AUA Best Practice Statement "The Evaluation of the Azoospermic Male", last reviewed in 2011, recommends "*testing for cystic fibrosis transmembrane conductance regulator abnormalities [that] should include at minimum a panel of common point mutations and the 5T allele*" (31). A consensus on the minimum number of variants for which to test is lacking. In cases of high risk to offspring, namely in men with CBAVD with negative *CFTR* panel testing and a *CFTR* carrier partner, complete gene

sequencing should be considered.

Current *CFTR* mutation testing is typically done by searching for panels of predetermined high-risk or high-frequency mutations. Most *CFTR* panels will include 30 to 96 of the most common mutations. Contrast this to the over 2,000 mutations identified in the CF Mutation Database (5). Our own institution's *CFTR* test offerings include a CF screen (32 mutations), CF expanded screen (161 mutations), CF intron 8 poly-T analysis, and CF complete rare mutation analysis or entire gene sequence. The common variants included in the 32-mutation screening panel including their relative frequency in those with CF are depicted in *Table 3* (35). Testing is performed via a multiplex polymerase chain reaction using specific primers for each genetic variant. Multigene panels at other laboratories may employ alternative methods such as multiplex ligation-dependent probe amplification or gene-targeted microarray (1).

Failure to identify a variant on *CFTR* mutation panel testing in a man with CBAVD should not be misinterpreted as ruling out a mutation. In a series of 64 men with CBAVD, 46% had detectable mutations missed by a 31-mutation panel which was decreased to 17% missed when including IVS8-5T analysis (9). The same study reported undetected mutations in up to 58% of men with idiopathic EDO using the panel and IVS8-5T testing. For purposes of reproductive planning, it should be assumed that all men with CBAVD harbor a *CFTR* abnormality, therefore negative testing only suggests the absence of the most common mutations. Expanded panels, entire gene sequencing, and thorough partner testing should be considered if the index of suspicion is high.

Diagnosis of *CFTR* mutations offers several potential benefits. Referral to a center specializing in CF care should be considered for a full assessment of CFTR dysfunction phenotypes. In patients with mild CF symptoms such as sinusitis not previously recognized to be *CFTR*-related, alternative therapeutic management options may be offered. Patient testing also provides important genetic information for siblings. Siblings of a man with CBAVD with biallelic *CFTR* mutations have a 75% chance of harboring at least one mutation inherited from their parents.

Partner testing also deserves special attention. When planning assisted reproductive techniques for men with known or suspected *CFTR* abnormalities, female partner screening should be offered (31,46). If the female partner is found to be a carrier, preimplantation genetic diagnosis is useful to determine risk of CF and CBAVD to future progeny. Genetic counselling should be recommended as

Table 3 Frequency of *CFTR* mutations in 32-mutation CF screening panel (note intron 8 poly-T analysis not included)

Mutation	Relative frequency (%)	Population with highest prevalence
F508del	66.0	Northern European
G542X	2.4	Ashkenazi Jewish, Spanish
G551D	1.6	English
N1303K	1.3	Italian
W1282X	1.2	Ashkenazi Jewish
R553X	0.7	German
621+1G>T	0.7	French-Canadian
1717-1G>A	0.6	Italian
R117H	0.3	–
R1162X	0.3	Italian
R347P	0.2	–
3849+10kbC>T	0.2	–
ΔI507	0.2	–
394delTT	0.2	Nordic, Finnish
G85E	0.2	–
R560T	0.2	–
A455E	0.1	–
1078delT	0.1	–
2789+5G>A	0.1	Spanish
3659delC	0.1	–
R334W	0.1	–
1898+1G>A	0.1	–
711+1G>T	0.1	French-Canadian
2183AA>G	0.1	Italian
3905insT	0.1	Amish, Acadian, Swiss
S549N	0.1	–
2184delA	0.1	–
3120+1G>A	–	–
V520F	–	–
3876delA	–	–
R347H	–	–
S549R	–	–

CF, cystic fibrosis.

early as possible in these cases, both for male and female partners.

Treatments and impact on male infertility

Following CF diagnosis, treatment is guided by efficacy-proven preventative and therapeutic algorithms. These treatments to date have largely targeted end-organ complications. Novel CFTR-modulator treatments aim to directly target CFTR protein dysfunction, effectively preventing downstream complications. Advancements in CF care have resulted in drastic improvements in patient survival and increase interest in assisted reproductive options (16).

Respiratory management is central to each CF patient's treatment plan. Individuals with CF may expect a sequence of inhaled medications including bronchodilators, hypertonic saline, dornase alfa, aerosolized antibiotics, and airway clearance techniques (1). Unfortunately, no current treatments exist to reverse the vasal atresia associated with CBAVD. Options for retrieval and utilization of sperm from men with *CFTR* mutations are discussed in a later article in this issue.

CFTR-modulator therapies, approved in 2012, are the first to directly target CFTR protein dysfunction rather than downstream organ involvement. These therapies currently fall into two approved categories (potentiators and correctors) with a third (amplifiers) being actively investigated. The theory behind potentiators and correctors is that altering the defects in the CFTR channel will allow some level of functional normalization leading to clinical benefit (47). Potentiators hold the CFTR chloride channel in an open position, thus any *CFTR* mutation that allows protein to reach the cell surface (Class 3 and some Class 4) will have improved ion conductance. Correctors have been designed to address F508del mutations specifically by binding to misshapen CFTR proteins, improving the three-dimensional structure and acting as chaperones to avoid elimination by the endoplasmic reticulum. Combining potentiator and single corrector therapies has shown significant but clinically limited improvement in patients with F508del mutations. Recent studies show that a combination of two correctors plus a potentiator, or triple therapy, provides a more significant clinical response (48). An additional class of modulator therapy, known as amplifiers, is now undergoing phase 3 studies. It is proposed that

amplifiers selectively increase the amount of abnormal CFTR protein thus providing additional substrate upon which correctors and potentiators can act (49).

It is interesting to speculate how CFTR-modulator therapy may affect male infertility in CF and CBAVD. When starting potentiators early (6–12 months of age), results suggest improved pancreatic function that was previously felt to be irreversible (50). Pancreatic insufficiency is hypothesized to occur in utero due to plugging of pancreatic exocrine ducts and autolysis. Although CBAVD is found in nearly all adult males with CF, it is less complete in children, raising the question whether infertility may lessen as modulator therapies become more pervasive and are initiated at earlier and earlier ages (40).

With FDA approval of the new gene therapy Zolgensma (onasemnogene abeparvovec-xioi) in May 2019 for infants with spinal muscular atrophy, we enter an exciting time in the treatment of genetic disorders altogether (51). Rather than responding to disease complications, future gene therapies carry the potential to prevent or reverse genetic changes leading to CF and CFTR-RD. With ground-breaking treatments, though, comes mind-boggling price tags. In the case of Zolgensma, the one-time treatment will cost over two million USD. The pathway to effective gene therapy for other diseases other than spinal muscular atrophy may not be smooth, though. CF and CBAVD represent phenotypic expressions of literally thousands of different *CFTR* mutations. Gene therapies for CF are in early clinical trials but have yet to show clinical effectiveness (1,52). It will be interesting to see how we manage the intersection of amazing technology, sky-rocketing costs, and swelling healthcare costs as future gene therapies and other CFTR-related treatments are developed.

Conclusions

CFTR abnormalities comprise a heterogeneous group of genetic mutations that have variable effects on protein quantity and functionality, manifested by inconsistent disease penetrance. Involved in the regulation of secretion viscosity, *CFTR* mutations can lead to CF and CFTR-RDs including male reproductive tract abnormalities and infertility. The severity of reproductive tract changes varies with both the genotype and the frequency of *CFTR* mutations. Studies have demonstrated gene abnormalities in almost all CF patients, approximately 80% of men with CBAVD, 46% of CUAVD patients, 33–47% of men with epididymal obstruction, and 17.5% of men

with oligospermia. A higher relative frequency of severe compared to mild *CFTR* mutations exists in men with CF trending progressively downwards in men with CBAVD, CUAVD, epididymal obstruction, and oligospermia. Men with CF and CBAVD have significantly higher chances of having children with classic CF, thus these men and their partners should be screened for *CFTR* mutations.

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