Unraveling the Role of CFTR Gene Mutations in Male Infertility: A Comprehensive Review of Insights, Clinical Evidence, and Future Directions

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Abstract

Men with cystic fibrosis are almost always sterile due to congenital bilateral lack of vas deferens but they are able to reproduce with assisted reproduction. Research has shown correlations between elevated frequencies of CFTR mutations and the following conditions: idiopathic ejaculatory duct obstruction (EDO), congenital bilateral absence of the vas deferens (CBAVD), epididymal blockage, and oligospermia. The cystic fibrosis transmembrane conductance regulator (CFTR) protein, which is present on the exocrine gland epithelium, regulates the viscosity of secretions by acting as an ion transporter. The CFTR gene is primarily responsible for mutations that cause congenital bilateral absence of the vas deferens (CBAVD). About 2-6% of male infertility subjects and up to 25% of obstructive azoospermia cases are caused by CBAVD. Studies have shown that 78% of CBAVD patients have alterations in the CFTR gene locus, with 53% having two mutations and 25% having one. This review explores the molecular mechanisms of male infertility caused by CFTR gene mutations. It also evaluates CFTR gene structure, function, clinical evidence and impact of these genetic anomalies on reproductive function. Although research has provided insight on these correlations, much more has to be discovered on the specific processes and how they interact in male infertility. More research is required to create specialized diagnostic tools and therapy strategies for males who experience infertility due to CFTR gene mutations. As long as male infertility continues to be studied further, the future prospects for affected men and couples will become more effective.

Key words: CFTR mutations, male infertility, congenital bilateral absence of vas deferens (CBAVD), obstructive azoospermia, diagnostic tools

Introduction

The CFTR gene, or cystic fibrosis transmembrane conductance regulator gene, is a key genetic component present on the long arm of chromosome 7. The specific site is 7q31.2, and CFTR has a total length of 250 kb. Its principal function is to encode the CFTR protein. Mutations in the CFTR gene cause cystic fibrosis (CF) and ultimately affect the male reproductive function. This gene codes for a protein that plays an important role in the body's natural salt, water, and mucus synthesis. Defective CFTR function causes these organs to produce thick, sticky mucus, which causes a variety of symptoms and consequences. Currently, over 2,300 CFTR gene variants have been systematically documented, with each showing a different level of prevalence [1],[2]. The most frequent mutation is a three-nucleotide deletion (delta F508), which results in a misfolded protein that is promptly destroyed by the cell. Other common mutations include G542X, W1282X, G551D, R117H, and N1303K. The intensity of CF symptoms as well as their consequences differ according to the type and combination of mutations present [3]. These changes will be experienced differently by each individual, with various degrees of severity and influence. Furthermore, the incidence of these mutations varies between populations and geographical regions. Some mutations may be more prevalent in certain ethnic groups or regions of the world [4] . Congenital bilateral absence of vas deferens, termed CBAVD, can have a significant impact on male reproduction since it is caused by abnormalities in the CFTR gene, which accounts for 1-2% of infertility in men. The vas deferens, which plays an important role in the movement of sperm from the testes to the urethra during ejaculation, is either completely absent or blocked in CBAVD. Hence, the lack of vas deferens in CBAVD impedes the transportation of sperm during ejaculation, even though sperm is produced normally in the testes. Males affected by CBAVD are generally sterile,

indicating their inability to naturally conceive children. This condition poses challenges to conception and adversely influences male fertility. The prevalence of CFTR mutations in CBAVD is markedly greater compared to non-CBAVD male infertility [5].

Due to the lack or obstruction of the vas deferens, the tube responsible for carrying sperm from the testes, cystic fibrosis (CF) in males may impair their ability to reproduce. However, developments in assisted reproductive treatments, such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), have made it possible for certain males affected by cystic fibrosis (CF) to achieve biological parenthood. Conversely, females with CF may encounter diminished fertility as a consequence of the thickening of cervical mucus, thus impeding the successful traversal of sperm toward the ovum. However, it is still possible for women with cystic fibrosis to get pregnant and have successful deliveries [6].

CFTR Gene Structure and Function

The CFTR gene, which stands for Cystic Fibrosis Transmembrane Conductance Regulator, carries a modular transmembrane protein that is essential for regulating ion and fluid balance across numerous epithelial cell membranes in the human body. Cystic fibrosis (CF) is caused by mutations in this gene, which has also been linked to male infertility. CFTR is a protein that passes through the cell membrane just once. It has an overall length of 27 exons, and the protein is made up of 1,480 amino acids and measures around 170 kilodaltons (kDa). Its structural layout involves the presence of several domains, each with unique functions. CFTR has two transmembrane domains known as TMD1 and TMD2, which penetrate the lipid bilayer [7],[8].

The domains include a vast number of alpha-helices that move through the membrane, enabling ion transport. CFTR contains two nucleotide-binding domains (NBDs), NBD1 and NBD2, which are located in the cytoplasm. These NBDs possess the responsibility for binding and hydrolyzing ATP (adenosine triphosphate), supplying the energy required for ion transport. The regulatory center, known as the R domain, is located between NBD1 and TMD2 and is critical in allowing the channel to open. The phosphorylation of the R domain controls CFTR activity, as seen in Figure 1. As a result of this, CFTR is activated in response to intracellular cAMP (cyclic adenosine monophosphate). When cAMP levels grow, protein kinase A (PKA) starts phosphorylating the R domain, which eventually leads to the channel opening. When active, CFTR acts largely as a chloride (Cl-) channel, allowing Cl- ions to cross cell membranes [7]. Mutations in CFTR result in the formation of thick and sticky mucus, which is a defining feature of CF. CFTR is also expressed in sweat glands, and mutations in this gene result in excessive salt loss through sweat, which is a diagnostic feature of CF. In addition to chloride, CFTR is involved in the transportation of bicarbonate and other ions in different tissues. This particular function has an impact on the pH and ion composition of secretions [8].

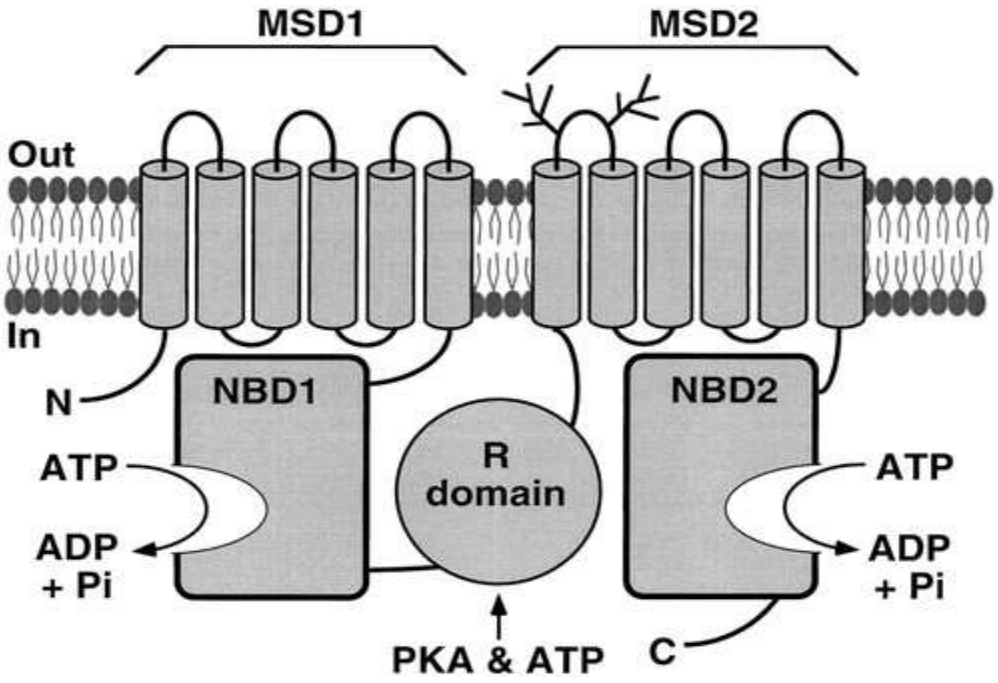

Fig. 1

Model illustrating the predicted domain structure of the transmembrane conductance regulator (CFTR) in cystic fibrosis. PKA, or cAMP-dependent protein kinase; R, or regulatory domain; NBD, or nucleotide-binding domain; and MSD, or membrane-spanning domain [9].

Types of CFTR genetic mutations

CFTR gene mutations are currently classified based on the underlying cause of malfunction, which includes improper protein translation, erroneous cell processing, or CFTR channel closure. These CFTR gene variants are roughly classified into six separate classes, each of which corresponds to a distinctive type of CFTR malfunction. The following are the several types of CFTR mutations [10],[3],[11]. The CFTR gene has 11.4% splicing mutations, 15.6% frameshift mutations, 39.6% missense mutations, and 8.3% nonsense mutations; 2.6% severe and 2.0% in-frame deletions or insertions; 0.7% promoter mutations; and 15.0% considered non-pathological variations [12].

Class I mutations: non-functional protein synthesis. Non-functional protein synthesis is caused by Class I mutations. This type of aberration is frequently caused by genetic abnormalities known as nonsense, frameshift, or splice-site mutations. These mutations cause messenger RNA (mRNA) transcripts to be prematurely terminated and the CFTR protein to be completely absent. G542X, W1282X, R553X, 621+G>T, and 1717-1G>A are some notable occurrences [10],[3]. The prevalence of class 1 mutations on a global scale stands at 10%, predominantly characterized by the presence of G542X and W1282X variants, which exhibit the most frequent occurrence and demonstrate severe phenotypic consequences [1].

Class II mutations: protein transport failure. This type of mutation causes improper post-translational processing of the CFTR protein, preventing accurate cellular localization and leading it to be destroyed prematurely by proteasomes. Prominent examples of this type of mutation include F508del, N1303K, I507del, and G85E. The F508del mutation is particularly notable since it is prevalent in around 50% of CF patients in a homozygous condition and in at least 90% of CF patients in a heterozygous state [10],[3]. The prevalence of class 2 mutations on a global scale stands at 70%, predominantly characterized by the presence of F508del and N1303K variants, which exhibit the most frequent occurrence and demonstrate severe phenotypic consequences [1].

Class III mutations: defective protein gating. The diminished channel activity that arises from these mutations is observed even when ATP levels are sufficient. A number of mutations affect the ATP-binding regions of the NBF (Nucleotide Binding Fold) domain, specifically NBO1 and NBO2. Certain variations of these mutations still show varied degrees of sensitivity to nucleotide binding. The most common class III mutation in Caucasian populations is the replacement mutation G551D in CFTR, which entirely removes ATP binding. Other CFTR mutations in the area that encodes the CFTR R domain, such as S549R, G551D, and G1349D, may also fall into this group [10],[3]. The prevalence of class 3 mutations on a global scale stands at 2-3%, predominantly characterized by the presence of G551D and R560T variants, which exhibit the most frequent occurrence and demonstrate severe phenotypic consequences [1].

Class IV mutations: reduced conduction of the channel. Despite the fact that the CTFR protein is appropriately produced and delivered to the cell surface, its ion flow rate and channel opening length are reduced compared to normal CTFR protein, even though chloride flows are created in response to cAMP stimulation. In Caucasian populations, the most prevalent class IV mutation is an amino acid substitution (R117H) that affects the CTFR protein. Additional examples of this type of mutation include R334W and D1152H [10], [3]. The global prevalence of class 4 mutations remains uncertain, accounting for less than 2% of cases. These mutations are predominantly represented by the R117H and R347P variants, which are the most commonly observed and are associated with mild phenotypic effects [1].

Class V mutations: decreased CFTR protein production. Certain classification systems do not include this class. It includes mutations that affect the integrity of mRNA as well as mutations that affect the stability of the fully produced CFTR protein (the latter being commonly categorized as a separate class known as class VI). Instances of such mutations include A455E, 2789+5G>A, and 3849+10kbC>T [10],[3]. The global prevalence of class 5 mutations remains uncertain, accounting for less than 1% of cases. These mutations are predominantly represented by the 3349+10kbC>G and IVS8-5T variants, which are the most commonly observed and are associated with mild phenotypic effects [1].

Class VI mutations: decreased CFTR stability. This course causes severe instability in the plasma membrane, which leads to increased endocytosis and lysosome degradation, as well as decreased recycling to the plasma membrane protein stability, including Phe508del when rescued by most correctors (rPhe508del), ultimately resulting in CFTR folding, moving, and functioning (almost) normally, but with a shorter membrane lifetime; notable examples include rPhe508del, 120del123, and Q1411X [10],[3],[13]. The global prevalence of class 6 mutations remains uncertain. These mutations are predominantly represented by the Q1114X variants, which are the most commonly observed and are associated with mild phenotypic effects [1].

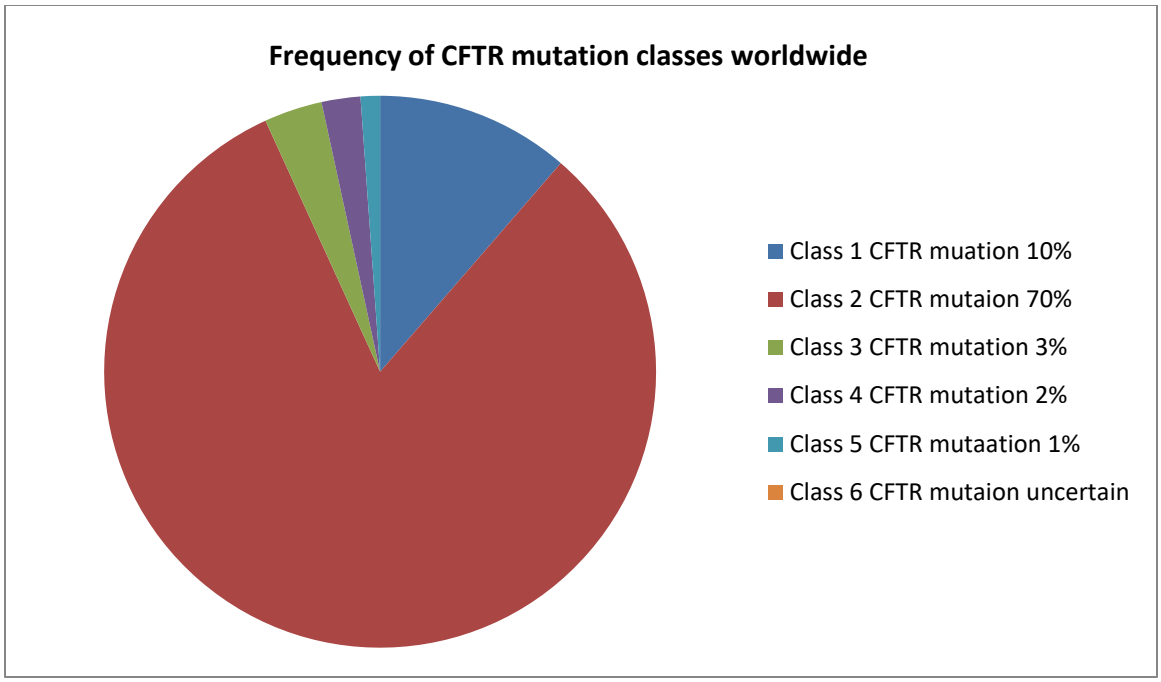

Fig. 2 : Pie chart that shows the prevalence of CFTR gene mutations on global scale

Efforts are presently underway to assess the functional effects and clinical severity of the discovered mutations, though the disease risk of many of them remains unknown. It has been discovered that mutations in class I–III cause more severe disease than mutations in class IV–VI. However, even among individuals with the same combination of mutations, the medical manifestations of cystic fibrosis (CF) might differ, presumably due to the action of gene modifiers. For example, in CF instances related to pulmonary disease, there is a moderate correlation between genotype and phenotype, whereas a slightly stronger correlation exists in CF types associated with pancreatic failure. Therefore, making judgments regarding the severity of CF in a single patient based simply on individual mutations is not recommended. Medical decisions should instead be based on patient indicators such as progression, respiratory health, and nutritional illness. However, the study and analysis of mutations can serve a valuable purpose in directing the initial treatment approach for certain individuals. This is particularly relevant due to the emergence of several novel therapeutic interventions that specifically target cystic fibrosis (CF) disease resulting from particular categories of CFTR mutations [10],[3],[11].

Clinical Manifestations of Cystic Fibrosis

The clinical manifestations of cystic fibrosis can exhibit considerable variation among individuals, encompassing common symptoms such as lung complications, digestive system issues, sweat gland dysfunction, reproductive problems, and other complications. The presence of dense mucus in the respiratory system can obstruct the airways, trap pathogens, and ultimately give rise to infections, inflammation, and respiratory failure. The accumulation of mucus in the pancreas can impede the release of digestive enzymes, culminating in malnutrition and inadequate growth, thereby causing gastrointestinal difficulties. In the context of the liver, the dense mucus has the potential to obstruct the flow of bile through the bile duct, consequently leading to liver disease. An elevation in the levels of salt in the sweat of individuals with cystic fibrosis is frequently employed as a diagnostic measure for the condition, thereby contributing to the dysfunction of sweat glands [8].

Due to the lack or obstruction of the vas deferens, the tube responsible for carrying sperm from the testes, cystic fibrosis (CF) in males may impair their ability to reproduce. However, developments in assisted reproductive treatments, such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), have made it possible for certain males affected by cystic fibrosis (CF) to achieve biological parenthood. Conversely, females with CF may encounter diminished fertility as a

consequence of the thickening of cervical mucus, thus impeding the successful traversal of sperm toward the ovum. However, it is still possible for women with cystic fibrosis to get pregnant and have successful deliveries [6]. Congenital bilateral absence of vas deferens, termed CBAVD, can have a significant impact on male reproduction since it is caused by abnormalities in the CFTR gene, which accounts for 1-2% of infertility in men. The vas deferens, which plays an important role in the movement of sperm from the testes to the urethra during ejaculation, is either completely absent or blocked in CBAVD. Hence, the lack of vas deferens in CBAVD impedes the transportation of sperm during ejaculation, even though sperm is produced normally in the testes. Males affected by CBAVD are generally sterile, indicating their inability to naturally conceive children. This condition poses challenges to conception and adversely influences male fertility. The prevalence of CFTR mutations in CBAVD is markedly greater compared to non-CBAVD male infertility [5].

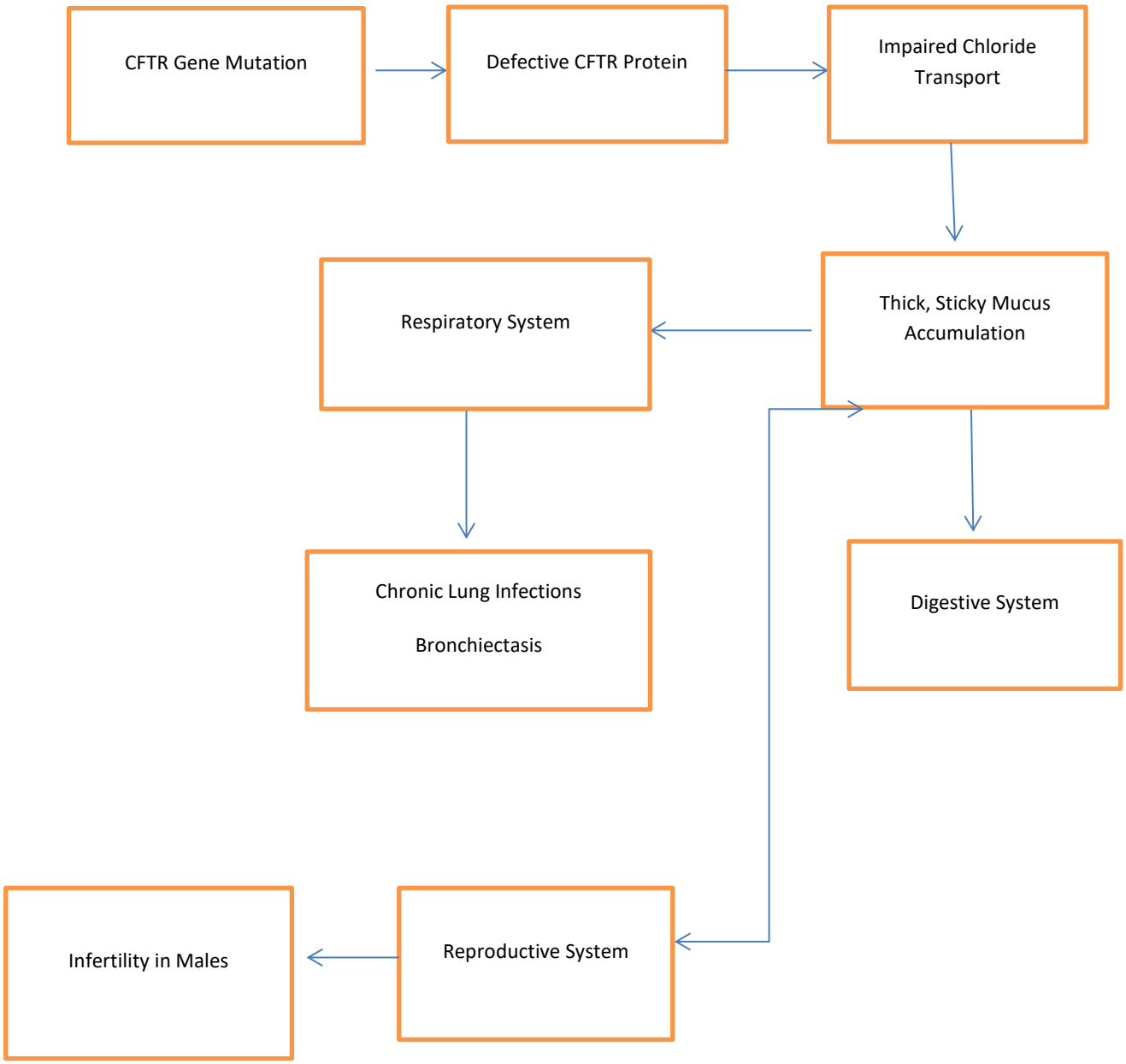

Fig. 2: Flowchart that shows the pathophysiology of CFTR gene mutation

Table 1: Frequency of CFTR gene mutations in different populations of infertile men diagnosed with CBAVD

Number of men diagnosed with CBAVD	Population	Frequency of CFTR gene mutations	Research
263	Chinese	1.90%	Fedder *et al.,* (2021)
125	Russian	39.2%	Chernykh *et al.,* (2023)
72	Russian	81.9%	Marnat *et al., (*2020)
34	Caucasian	45.5%	Mafruchati *et al.,* (2022)
56	Austria	78.57%	Rudnik et al.,(2021)

Molecular Mechanisms of Male Infertility due to CFTR Gene Mutations

Several molecular mechanisms contribute to male infertility in individuals with CFTR mutations, shedding light on the intricate interplay of biological processes. CFTR mutations hinder ion transport in the male reproductive system, particularly in the epididymis and vas deferens, which disrupts the regulation of chloride and bicarbonate ions, leading to changes in seminal fluid composition, duct blockages, and impaired sperm movement and maturation, thereby compromising their ability to fertilize the egg [14]. Reduced chloride transport can also increase the viscosity of seminal fluid, hindering sperm movement through the female reproductive tract and reducing the chances of successful fertilization [15]. Mutations in CFTR can impact male fertility via mucus abnormalities. Changes in mucus quantity and quality can affect fertility. Inadequate mucus production can reduce sperm motility and their ability to travel through the female reproductive tract. Mutation impacts bicarbonate ions in mucus, which help sperm survive and function. Suboptimal pH conditions due to CFTR mutations affect sperm motility and viability. Mucus abnormalities can cause mucus plugging, blocking ducts and passageways. This can cause infertility by preventing semen and sperm flow. Thick mucus can obstruct the epididymis and vas deferens, requiring assisted reproductive techniques for conception [16],[17].CFTR mutations can cause dysregulation of ion transport and mucus production, leading to chronic inflammation in the male reproductive tract. These changes create a more susceptible environment for infections and irritation. Pathogens or aberrant mucus composition can stimulate immune cells like neutrophils and macrophages. Immune cells release inflammatory mediators (such as chemokines and cytokines) in response to infections or toxins, which play an important part in the inflammatory response [18]. Chronic inflammation can damage the reproductive tract, affecting sperm production, transport, and storage. Inflammatory responses can lead to scar tissue, blockages, and fibrosis in the reproductive ducts, causing infertility. Chronic inflammation can also impact sperm health and quality by inducing the release of reactive oxygen species that damage sperm DNA and affect their motility and viability [19].

Oxidative stress is caused by an imbalance between the synthesis of reactive oxygen species and their removal from the organism. It causes serious genetic impairments like chromosome damage and DNA discontinuities. CFTR mutations hinder the production and regulation of antioxidant molecules (superoxide dimutase and glutathione), thereby impairing cellular protection. Inadequate antioxidants lead to elevated ROS levels, damaging sperm cells and affecting their function by attacking their lipids, proteins, and DNA. Oxidative stress can negatively impact the function of sperm cells, including their ability to undergo capacitation, acrosome reactions, and fertilization. Elevated ROS also impact the composition of the seminal fluid, disrupting its composition. Increased oxidative stress can result in DNA fragmentation, lipid peroxidation, and apoptosis in sperm, potentially affecting embryonic development [20],[21].

Table 2: Frequency of CFTR gene mutations in different populations with male infertility conditions

Sample Size	Population	Condition of infertility	Frequency of CFTR gene mutations	Research
76	Chinese	Obstructive Azoospermia	46.5%	Wang *et al.,* 2024
32	Egypt	Obstructive Azoospermia	12.5%	Toson *et al.,* 2023
30	European	Severe Oligoospermia	3.33%	Cantineau *et al.,* 2021
200	Iran	Oligoasthenospermia	7%	Jafari et al., 2022
46	China	Non obstructive Azoospermia	8.7%	Liu *et al.,* 2023

Clinical Evidence of the Link between the CFTR Gene Mutation and Male Infertility

Studies have conclusively shown that male infertility and mutations in the CFTR gene are related. Genetic testing of individuals with infertility issues has identified specific CFTR gene mutations in affected individuals. Numerous investigations carried out throughout different nations have furnished proof that the pathophysiology of male infertility, namely in those suffering from obstructive azoospermia, is directly associated with CFTR gene mutations [22]. A study conducted in 2023 measured the prevalence of the L138ins variants in the CFTR gene by studying 6033 infertile males from Russia. According to the study, 3.9% of patients had pathogenic variations in the CFTR gene. The most prevalent variants were F508del (61%) and CFTRdele2.3 (7.1%), with the L138ins variant being observed in 0.28% of patients [13]. investigated the possible role of five frequent variants in the CFTR gene (ΔF508, G551D, G542X, W1282X, and R117H) in 32 infertile males from Egypt. Four out of the 32 individuals had heterozygous CFTR mutations (12.5%). ΔF508 and R117H were the only two mutations that tested positive, with incidences of 9.4% and 3.1%, respectively.

Some studies conducted by researchers indicate that the CFTR gene mutation can also be detected in compounds heterozygous with other CFTR gene mutations. A study conducted in 2022 examined the location of Y chromosomal microdeletions and CFTR gene mutations in individuals with extremely severe oligozoospermia (200) [23]. According to the results, AZFa (3%) was deleted in 6 patients, followed by AZF b (2.5%), AZFc (1.5%), AZF a/c (1.5%), and AZF b/c (2%). The most prevalent CFTR gene mutation (4.5%) was F508del; G542X and W1282X were found to be 1.5% and 1% of cases, respectively. It was discovered that one patient had heterozygote F508del and AZFa microdeletion, while another patient had F508del and AZFb microdeletion. In one instance, F508del was shown to be compound heterozygous with G542X, whereas in the other patient, it was revealed to be W1282X. The distribution of mutations in the CFTR gene, the M470V polymorphism, and the IVS8 polyT was examined by [23]. The goal of the study was to identify a novel CFTR gene mutation in 200 infertile Iranian males. F508del (4.5%) was the most prevalent mutation in the CFTR gene. At 1.5% and 1%, respectively, G542X and W1282X were found. R117H and N1303K were found in 0.5% of instances. In one case, F508del was identified as a heterozygous compound with G542X, and in the other, with W1282X. A novel mutation was discovered in the aforementioned exons, and the PolyT test revealed statistical differences in some genotypes.

Table 3: Summary of research conducted to show the prevalence of most common CFTR gene mutations that result in infertility

Sample Size	Common Mutation	Prevalence of CFTR gene mutation	Research
200	F508del G542X W1282X	4.5% 1.5% 1%	Jafari *et al.* (2023)
6033	F508del CFTRdele2.3 L138ins	61% 7.1% 0.28%	Chernykh *et al.* (2023)
91	F508del	3%	Stela *et al.* (2022)
200	F508del G542X W1282X N1303K R117H	4.5% 1.5% 1% 0.5% 0.5%	Jafari *et al.* (2022)
32	F508del R117H	9.4% 3.1%	Toson et al. (2023)

Treatment Approaches

Individuals with CFTR gene mutations can achieve fertility through assisted reproductive techniques such as intracytoplasmic sperm injection (ICSI) and testicular sperm extraction (TESE), including microdissection TESE. ICSI is highly effective for severe male infertility, even with a limited quantity or quality of sperm. TESE procedures provide options for individuals with genetic mutations affecting sperm production. TESE involves the surgical removal of testicular tissue to extract sperm directly from the testes. Microdissection TESE allows the extraction of healthier sperm by identifying areas with higher sperm production. These techniques overcome issues related to sperm production caused by genetic mutations, providing viable sperm for use in assisted reproduction [24]. During in vitro fertilization (IVF), pre-implantation genetic diagnosis (PGD) is utilized to find genetic flaws in embryos prior to implantation. It allows the selection of embryos free from genetic disorders, including CFTR gene mutations and Yq microdeletions. For couples affected by conditions like CBAVD, which CFTR gene mutations can cause, PGD is useful. By choosing healthy embryos, it lessens the chance of genetic abnormalities spreading and raises the possibility of a successful pregnancy [25], [26]. CFTR modulator medicines, which address genetic abnormalities caused by CFTR mutations, are the result of advances in pharmacology. By enhancing sperm motility and quality, these treatments hold great promise for treating male infertility. For those with CFTR-related infertility, this strategy offers fresh hope as it moves toward customized therapy [1].

Future Directions

The area of study on the relationship between male infertility and CFTR mutations is complex and still evolving. Even though we now understand this association better, more research is still required to elucidate the processes and provide more useful diagnostic and treatment options. The molecular and cellular processes underlying the effect of CFTR mutations on male fertility will be further investigated. This might help guide customized treatment regimens and help identify which genes are most strongly associated with infertility [1]. It will help to discover CFTR mutations and their effect on male fertility by developing more precise and sensitive diagnostic procedures. Proteomic profiling, next-generation sequencing, and advanced imaging techniques may be used to assess the structure and function of sperm [27]. It is crucial to find biomarkers in blood or semen that are capable of accurately foreseeing male infertility brought on by CFTR mutations. These indicators may facilitate the diagnosis procedure and shed light on the severity of the ailment [28]. To mitigate the effect of CFTR mutations on male fertility, it is crucial to look into potential pharmacological therapy. This could entail creating medications that specifically target CFTR mutations or processes related to the development and functionality of sperm [29]. It is essential to investigate the viability of using gene editing methods, such as CRISPR-Cas9, to resolve CFTR mutations in testicular cells or sperm. Find gene therapy strategies to help the male reproductive system's CFTR function effectively [30]. It is imperative to investigate the correlation between CFTR mutations and environmental or lifestyle factors that either increase or alleviate male infertility. This could involve elements like exercise, nutrition, and toxic exposure [1]. To evaluate the long-term implications of CFTR-related infertility and the impacts of treatment options on reproductive outcomes and general health, conduct longitudinal research [27]. Provide informational materials and support systems for people and couples coping with CFTR-related male infertility. Address the emotional and psychological effects of infertility [31]. To enable a complete approach to diagnosis and treatment, promote cooperation between researchers, doctors, geneticists, and reproductive specialists [1]. By proceeding in these new directions, scientists are hoping to help individuals and couples cope with male infertility caused by CFTR mutations by offering more effective diagnostic tools, individualized treatment plans, and better outcomes.

Conclusion

Male infertility affects a significant portion of couples worldwide who are attempting to conceive and is a major issue that is occasionally disregarded. CFTR gene mutations play a significant role in male infertility. CFTR mutations can take many different forms, but they frequently result in obstructive azoospermia by impairing the vas deferens' ability to grow or function. Furthermore, because of their involvement in ion transport throughout the male reproductive tract, these alterations may potentially disrupt the quality and motility of sperm. It has been demonstrated that the particular CFTR mutation and its severity influence the degree of male infertility, with some people experiencing more severe difficulties than others. For couples facing male infertility due to CFTR gene mutations, innovations in assisted reproductive technologies such as intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF) offer an illuminating sign of hope despite the challenges. Further study is necessary to clarify the precise mechanisms at which

they function and establish targeted medicines that can enhance the chances of conception for those impacted by CFTR gene mutations as we continue to explore the subtleties of this relationship.

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