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Cystic Fibrosis: CFTR, Complications, and Prospective Therapies

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Introduction

One of the most common and well-known genetic disorders is Cystic Fibrosis (CF). CF is a fatal autosomal recessive disorder that affects many organs including the lungs, pancreas, and sweat glands [1]. It is caused by a defective gene that leads to the accumulation of a thick mucus layer that covers the body's airways and pancreas. This gene is known as the CFTR gene, which encodes for a protein called the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). The CFTR is a transmembrane channel located in epithelial cells that controls the movement of chloride and bicarbonate ions into and out of the cell. If both CFTR genes are defective, there will be no functioning CFTR protein, and the patient will exhibit the symptoms of CF [4]. A mutation within the CFTR gene can cause defects in a number of regions including synthesis, trafficking, stability, function, and regulation of the ion channel [5].

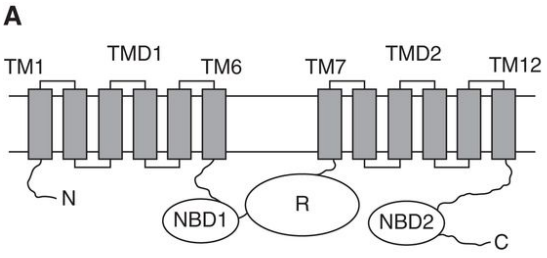
Cystic Fibrosis was not recognized as a separate disease until 1938. Before this time, it was grouped together with Celiac disease [6]. CF occurs in about one in 2500 Caucasian live births. However, this number only accounts for the defective genes found within homozygous individuals. Many more people are born each year who carry a single defective CFTR gene without even being aware of it [5]. Over the years, a greater understanding of the CFTR and CF as a whole has led to many promising treatments and possible cures. There is hope that as CF becomes more widely understood there will be a greater support from the general community, hopefully leading to more extensive research focused on finding a cure.

CFTR Structure

The CFTR protein is a chloride channel found within the apical membrane of epithelial cells. CFTR is a member of the superfamily of ATP-binding-cassette (ABC) transporter proteins and is currently the only member known to function as an ion channel [7]. A total of 48 ABC

transporter proteins have been discovered in the human genome to date. The ABC proteins were named based on the discovery that all the members of the superfamily contain two homologous nucleotide-binding domains (NBDs) [8]. Along with the two cytoplasmic NBDs, ABC transporters contain two transmembrane domains (TMDs) which are connected to the NBDs. Unlike other ABC transporters, the CFTR also contains a regulatory (R) domain that is found in between the two halves of the protein. Therefore, there are two semi-symmetrical halves on either side of the R domain, each containing one NBD and one TMD [3]. This gives the CFTR the general organization of TMD1 – NBD1 – R – TMD2 – NBD2. The TMDs each contain 6 membrane-spanning alpha-helices, which creates a protein that crosses the membrane a total of 12 times [2]. The general structure of CFTR can be found in figure 1.

Figure 1



This image depicts the general structure of CFTR. There are two TMDs with six membrane-spanning domains each. The regulatory (R) domain is located in the center of the protein, and there are two NBDs.

[3]

ABC transporters are typically considered ATPases because they hydrolyze ATP in order to move substances against their concentration gradients. While most ABC transporters hydrolyze ATP at both ATP-binding sites, some, including the members of the ABCC subfamily of which CFTR is a part, only hydrolyze ATP at one site [8]. Within the ABCC subfamily, CFTR is the only ATP-gated ion channel that allows for the passive diffusion of chloride ions. All other members of the ABCC subfamily hydrolyze ATP in order to move large hydrophobic anions out of cells. Studies have shown that ABC transporters exist in two different

conformations. For the ABC transporters, including CFTR, that export molecules from cells, the substrate-binding site on the TMDs is open to the cytoplasmic side of the plasma membrane when ATP is absent. On the other hand, when ATP binds, dimerization of the NBDs occurs along with a conformation change within the TMDs. This leads to the opening of the substrate-binding site to the extracellular side of the membrane [3].

The R domain of the CFTR separates the NBD1 and TMD2 (fig.1). This domain seems to be unstructured, but it can be distinguished by a set of phosphorylation sites that are highly conserved. The R domain contains 200 residues and is the least conserved region within the entire CFTR protein sequence. However, the positions of the phosphorylation sites and the pattern of order-disorder within the protein sequence seem to be highly conserved. This proves that the R domain is functionally significant within the CFTR [8].

Since the CFTR protein is an ion channel, it must contain a pore somewhere within its structure. However, only a few studies have been carried out to determine the residues involved in the anion pore. Therefore, there is still uncertainty about what residues play an important role in this structural unit. Most research has been focused on the sixth membrane-spanning helix in TMD1. The cytosolic process of this structure connects TMD1 with NBD1. Since the CFTR has an anion-selective pore, it is predicted that the sixth membrane-spanning helix contains at least three basic residues. Arg 334 and Lys 335 are found towards the extracellular end while Arg 347 is found about four helical turns away closer to the cytoplasmic end. The Arg 347 makes a salt bridge with an Asp found in the membrane-spanning helix 8. The positive charges on both Arg 334 and Lys 335 aid in the entry of anions into the CFTR pore. The CFTR pore selects poorly among small anions. This implies that the CFTR pore is relatively featureless and has no region that makes an intimate contact with the chloride ions [2].

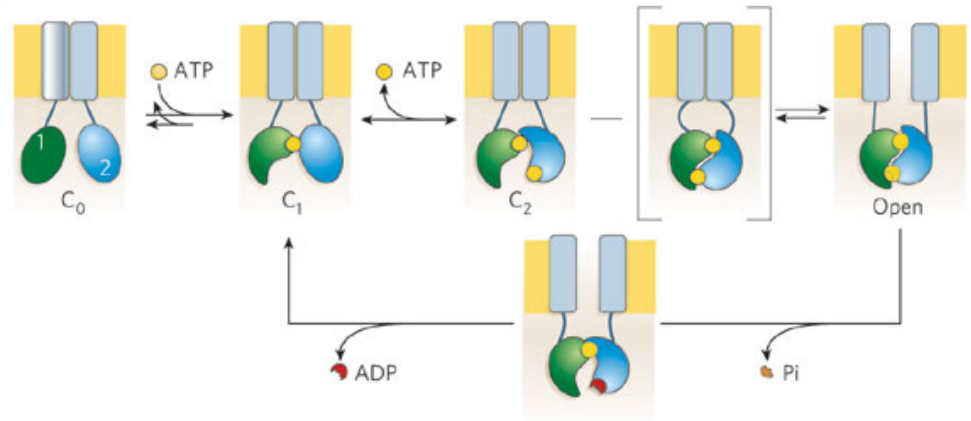
CFTR Function and Regulation

As stated before, the CFTR protein is the only member of the ABC transporter superfamily to function as an ion channel and helps to mediate the anion conductance across epithelial membranes. This channel is required for both the absorption and secretion of chloride ions. The CFTR also functions to move bicarbonate across epithelial membrane in both directions both by transporting bicarbonate itself and by stimulating other chloride-bicarbonate exchangers. Although both chloride and bicarbonate flow through the CFTR based on their concentration gradients, CFTR is considered an active transporter because it requires ATP hydrolysis to regulate the opening of the channel [8]. The function of the CFTR as an ion channels has sparked a popular theory as to why CF is so prevalent in the human population. According to this theory, CFTR mutations became prevalent because those who had one mutated CFTR gene had a greater chance of escaping death from the hands of Cholera. Decreased CFTR channel activity reduces diarrhea by retaining chloride (this works) in the epithelial cells surrounding intestinal cavities. Therefore, since people with the CFTR gene had a selective advantage, the defective gene was passed on leading to the prevalence of CF [9].

Knowing the overall function of the CFTR, the next question is how the CFTR protein carries out this function. The CFTR has ATP-binding sites on both of its NBDs. When ATP binds to both of these sites, the CFTR channel opens. However, unlike other classic ligand-gated ion channels, the ATP used by the CFTR is consumed in order to close the channel (fig. 2). Also, unlike many other ABC transporters, only one NBD of the CFTR hydrolyzes ATP. The NBD1 domain is degenerated and has many substitutions that render it incapable of hydrolyzing ATP though the ATP can still bind to it. While ATP gates the CFTR channel, it cannot carry out this job effectively without the help of a magnesium ion. Therefore, ATP does not bind to the CFTR,

MgATP does. The magnesium helps ATP bind to CFTR by interacting with an Asp residue. The magnesium is also essential for the hydrolysis of ATP which drives the closure of the CFTR gate [3].

Figure 2



This figure shows the hypothesized ATP-dependent gating cycle of CFTR. In the figure, the green shape is NBD1 and the blue shape is NBD2. Initially, the CFTR channel is closed. However, once ATP binds to both NBDs, the channel opens. When ATP is hydrolyzed by NBD2, the channel reverts back to a closed state. This is a simplified diagram that does not show the full details of CFTR gating cycle. A more detailed diagram would show that when ATP is absent, the substrate-binding site is open to the cytoplasmic side of the plasma membrane instead of the entire channel being closed. When ATP is bound to both NBDs, the substrate-binding site is also open to the extracellular side of the membrane fully opening the channel.

[2]

It is clear that ATP binding and hydrolysis is important for the opening and closing of the CFTR gate, but the mystery is how this occurs. It has been found that conformation changes within the NBDs are coupled with conformational changes within the membrane-spanning domains. These conformational changes shift the equilibrium between an open and closed CFTR pore [8]. Evidence has been found that the binding of ATP to CFTR leads to the formation of a NBD dimer. This dimer formation is then linked with the opening of the CFTR gate. It was then

hypothesized that that hydrolysis of ATP would lead to the dissociation of the dimer and therefore the closing of the gate [10].

Although this theory was popular, there has been recent evidence that disproves it. A new theory hypothesizes that there are both two open and two closed states for the CFTR gate. This was shown by the fact that the CFTR gate remains open for varying amounts of time depending on when pyrophosphate (PPi) is applied after the removal of ATP. When PPi is applied right after the removal of ATP, the channel stays open for approximately 30 seconds. However, if PPi is applied several minutes after the removal of ATP, the gate will only stay open for 1.5 seconds [11]. In this new theory, when the ATP bound to NBD2 is hydrolyzed, the CFTR channel closes into a partial dimer where the NBD remains partially closed. This prevents ATP from dissociating from NBD1 but allows the small anions to flow through the channel and a new ATP molecule to bind to NBD2. Based on this theory, the opening and closing of the CFTR gate is regulated by the formation of a NBD dimer and the partial separation of this dimer [12].

The gating of the CFTR protein is also regulated by phosphorylation of the R domain by protein kinase A (PKA). As mentioned earlier, the R domain contains approximately 200 residues and connects the NBD1 and TMD2. Within the R domain, there are multiple sites for phosphorylation by PKA. So far, six sites have been found in CFTR in living cells while eight have been found in isolated CFTR [2]. PKA phosphorylation of CFTR is the best understood mechanism of regulation for the CFTR channel. However, other mechanisms of regulation have been discovered that are less understood. For example, it has been found that protein kinase C increases CFTR activity while AMP kinase inhibits CFTR. Since these mechanisms are not well understood at this time, I will focus on regulation by PKA [3].

CFTR channels that are unphosphorylated typically are less active than channels that have been phosphorylated. The consensus theory is that an unphosphorylated R domain inhibits the opening of the CFTR channel, while phosphorylation of the R domain relieves this inhibitory effect and allows the channel to open [3]. Currently, the mechanism for how phosphorylation of the R domain controls channel gating is not understood. There are no other ABC proteins that have R domains, and the R domain of CFTR does not show any homology to other proteins. Therefore, there is no previous knowledge to be drawn from in order to understand the mechanism of regulation. Also, the conformation of the R domain and its location within the CFTR channel are currently unknown [2].

There are three main proposed mechanisms for the regulation of CFTR by PKA. Since there are multiple PKA sites in the CFTR, these mechanisms are not mutually exclusive. The first proposed mechanism is that the phosphorylation of the R domain regulates NBD dimerization [3]. It was found that split CFTR proteins could be cross-linked together at the NBD areas by adding cysteine amino acids to the dimer interface. Subsequently, it was discovered that PKA phosphorylation enhanced the efficiency of this cross-linking [13]. Another study supported this mechanism by structurally analyzing a fragment of the R domain of CFTR with NMR. The R domain fragment bound to NBD1 with most of the contact areas found near known PKA sites. The phosphorylation of this fragment decreased its ability to bind to NBD1. It was then proposed that CFTR function is inhibited when the R domain is unphosphorylated because it binds to the NBD1. This could in turn prevent the formation of the NBD dimer. The phosphorylation of the R domain would relieve this inhibition by reducing the affinity for NBD1, therefore allowing NBD dimer formation and opening of the CFTR channel [14].

The second proposed mechanism argues that the R domain also regulates CFTR channel function independently of the NBD dimerization. This regulation is thought to occur from physical interactions between the R domain, the TMDs, and their extensions within the cytoplasm. For example, it is thought that parts of the R domain interact with the cytosolic loops of CFTR when phosphorylated. This interaction then affects the conformation changes within the cytosolic loops that underlie CFTR gating.

The third mechanism proposes that phosphorylation of PKA sites in NBD1 affects the interactions between NBD1 and TMD. Although most PKA sites are found within the R domain, at least two other sites have been located within the NBD1. These regions within the NBD1 are called the regulatory extension and the regulatory insertion [3]. It was found that interactions between these specific regions and NBD1 are inhibited by phosphorylation. A binding interaction was also discovered between NBD1 and a peptide that only occurs when the regions in NBD1 are phosphorylated. It is thought that this peptide mediates the coupling of NBD1 and TMD1 [15]. From these three proposed mechanisms of regulation, it can be seen that there likely are multiple ways that PKA phosphorylation affects CFTR channel regulation.

CFTR Synthesis and Trafficking

The CFTR gene has approximately 180,000 base pairs that can be found on the long arm of chromosome 7. This gene encodes the 1,480 amino acids that make up the CFTR. In order for CFTR to function properly, it must be correctly synthesized, folded, and transported to the apical membrane of the cell. This path from the nucleus to the plasma membrane requires many proteins found in different compartments throughout the pathway. Along the way, the CFTR protein must pass stringent quality control which weeds out any misfolded protein that could cause damage to the cell or simply fail to function properly [16]. There are also quality control

systems that function throughout the life of the CFTR in order to remove proteins that might become damaged. All of these quality control systems function for membrane and secretory proteins. However, it has become apparent that the CFTR protein has more difficulty than other proteins in passing these rigorous tests and leaving the endoplasmic reticulum (ER). For example, other ABC transporters such as P-glycoprotein and MRP1 are able to mature and leave the ER at close to 100% efficiency. On the other hand, only approximately 33% of CFTR folds correctly and is allowed to leave the ER [8].

The first step in creating a fully functioning CFTR begins within the nucleus, where the CFTR gene is transcribed into mRNA. Once transcription is complete, the mRNA leaves the nucleus and travels to a ribosome which attaches to the ER. When the mRNA docks on the ribosome, tRNA carries amino acids to the ribosome and allows for the translation of CFTR [16]. While the polypeptide is being translated, it is also being pushed into the ER through a pore called the translocon. When the ribosome docks onto a translocon, it becomes known as ribosome-translocon complex (RTC). The RTC functions to synthesize, fold, orient and integrate the transmembrane (TM) sections of the CFTR into the membrane of the ER. The function of the RTC is controlled by specific sequences within the nascent polypeptide chain that is being translated [17].

While the CFTR polypeptide chain is being translated, it is actively being folded. CFTR is a membrane protein containing both membrane and cytosolic domains. The folding process for CFTR is complex because both the membrane and cytosolic domains must be correctly incorporated in order for the protein to function properly. This complex process follows two general sets of folding rules. The first rule is based on the hydrophobic effect. Within the cytosol of the cell, nonpolar residues are pushed towards the interior of the protein while the polar

residues are left on the outside of the protein. The second rule dictates the folding of proteins within the bilayer of the ER where the hydrophobic effect is virtually absent. This folding process is based on two main steps. First, polypeptide segments of 18-25 residues that are sufficiently hydrophobic are inserted into the ER membrane. Once these segments are in the membrane, they fold into a more stable TM alpha-helix. By folding into an alpha-helix, the free energy needed to bury the polar residues is minimized. The second step of this process is further folding. The various alpha-helices bind to each other in order to adopt a tertiary and/or quaternary structure [5].

While the CFTR is folding within in the ER, it receives help from proteins known as chaperones. The chaperones are important for the correct folding of CFTR and preventing aggregation [5]. The first and most important cytosolic chaperone found to complex with CFTR was Hsp70 [18]. The Hsp70 chaperone works alongside other chaperones and co-chaperones to assist in the correct folding of CFTR. For example, a co-chaperone known as Hdj-2 helps to reduce aggregation during the early stages of CFTR assembly possibly by recruiting Hsc70 [8]. Another major chaperone detected early on was calnexin [19]. Calnexin is located within the ER and has both a luminal lectin domain and a polypeptide-binding site. These two areas allow calnexin to interact with the immature CFTR and aid in its proper folding. It is thought that chaperones detect any exposed hydrophobic regions located on the outside of the protein [5].

After folding in the ER, the CFTR must pass through a quality control system known as the endoplasmic reticulum-associated degradation (ERAD) process. ERAD for CFTR typically involves the ubiquitin proteasome system (UPS) for which CFTR is a substrate [16]. In this system, misfolded proteins are polyubiquitylated and degraded by a proteasome. Ubiquitin is a small protein made up of 76 amino acids. If a protein is targeted for degradation, ubiquitin

covalently attaches to lysine residues on the CFTR. Ubiquitylation requires three types of enzymes which are the E1 ubiquitin activating enzymes, the E2 ubiquitin conjugating enzymes, and the E3 ubiquitin protein ligases. The ubiquitylation process begins with the activation of E1 through hydrolysis of ATP. This creates an activated ubiquitin which is then transferred to an E2 active site. An E3 ligase then covalently binds the activated ubiquitin to a lysine on the protein. Further ubiquitin molecules can then be linked together to form a polyubiquitin chain. Once the misfolded CFTR has been polyubiquitylated, it is removed from the ER membrane and targeted for degradation by the 26S proteasome in the cytoplasm. The CFTR must have at least four ubiquitin molecules in order to be degraded [20].

Misfolded CFTR proteins are detected by two separate systems during the folding process. One system detects defects in the cytosolic parts of the CFTR while the other system detects defects within the ER membrane. Ubiquitylating proteins seem to work with specific factors to detect misfolding such as the Hsp70 in the cytosol and Derlin-1 within the ER membrane. Hsp 70 detects mutations within the cytosolic region including the NBD1, NBD2, and R domain. It is currently thought that Hsp70 keeps the CFTR soluble until the E3 ligase CHIP binds with the Hsp70. The Hsp70-CHIP complex then redirects the CFTR to the degradation pathway. Within the ER membrane, Derlin-1 seems to be the main factor detecting mutations. Derlin-1 also seems to participate in the retro-translocation of CFTR from the ER so that it can be sent to the 26S proteasome for degradation [20]. ERAD within cells is an extremely complicated process that requires numerous factors and proteins that all interact with each other in complex pathways. This stringent system makes it nearly impossible for imperfect CFTR channels to make it to the membrane even if they could still function. However, without this system, our cells would be in danger of destruction from the aggregation of misfolded proteins.

If the CFTR protein is folded correctly within the ER, it is sent to the Golgi. This relocation requires the coat protein complex II (COPII) found within the ER membrane. The COPII helps to maintain the correct CFTR structure and location, conformation, and protein-protein interactions. Once within the Golgi, the final processing for the CFTR is carried out where the CFTR replaces a mannose-enriched side chain with a mature complex oligosaccharide side chain. When the CFTR is fully mature, it is moved from the Golgi to the apical membrane via clathrin-coated vesicles. After CFTR is situated in the plasma membrane, it has a half-life of about 12 to 24 hours. The CFTR is then internalized by clathrin-coated endosomes. Another quality control system exists to detect poorly functioning CFTR within the membrane. The CFTR is first recognized by Hsc70 which leads to ubiquitylation. The protein is then internalized into an endosome and either sent back to the plasma membrane or degraded within a lysosome [16].

CFTR Mutations

Over a thousand different mutations of the CFTR gene have been discovered that can lead to the cystic fibrosis phenotype. These mutations have been found in various locations such as the coding sequence for CFTR and the mRNA splice signals. The different mutations have been sorted into different classes based on the mechanism through which they lead to the disease state [21]. Mutations caused by deletions, frameshifts, and nonsense mutations that lead to a prematurely truncated CFTR are grouped into the class I mutations. Class II mutations generate proteins that are unable to pass through the intracellular trafficking process, even though many would be able function to some extent if they reached the plasma membrane. Class III mutations generate full length proteins that exhibit little or no channel activity once they reach the membrane due to disordered regulation. Class IV mutations result in proteins with slightly

reduced channel function, and therefore create a less severe phenotype of cystic fibrosis. Class V mutant proteins are fully functional but are expressed within cells at a reduced level, and class VI mutants are expressed at a normal level but seem to be less stable within the plasma membrane. Mutant classes I, II, and III lead to the most severe phenotypes because there is little or no channel activity associated with these mutations [20].

The most common CFTR mutation is the ΔF508 which accounts for 90% of all the mutant CFTR alleles. It is also responsible for two thirds of diagnosed CF cases [22]. The ΔF508 mutation results from the deletion of a phenylalanine at position 508. It was found that cells with the ΔF508 failed to produce fully glycosylated CFTR proteins; the CFTR is not fully processed and is degraded by the UPS before reaching the apical membrane. Therefore, the ΔF508 mutation is classified as a class II mutant [23]. A small amount of ΔF508 CFTR proteins are able to bypass the ERAD system and reach the membrane, however the proteins are unstable and function poorly due to endocytosis and degradation by the UPS [5]. The ΔF508 CFTR has also been discovered to be temperature-sensitive. At lower temperatures, ΔF508 CFTR starts to act more like wild-type CFTR, with channels reaching the membrane and functioning correctly [24].

The ΔF508 mutation occurs within the NBD1 of CFTR. It has been found that the backbone structure and thermodynamic stability of ΔF508 CFTR are similar to those of wild type CFTR [25]. However, the ΔF508 mutation may cause both kinetic and thermodynamic folding defects within the NBD1. During the folding process, CFTR employs a cooperative folding mechanism. This implies that the folding energetics of the wild type NBD1 is stabilized by coupled domain folding, but this coupled domain folding is impaired in the ΔF508 NBD1. Due to this impairment, the assembly of the interface between NBD1 and both of the MSDs is also impaired, which destabilizes the conformations of the MSD1, MSD2, and NMD2 [26]. The

incorrectly folded NBD2 acts as one of the signals targeting the CFTR for degradation and therefore accounts in part for the failure of ΔF508 CFTR to reach the apical membrane and function properly [22].

Another common mutation is the G542X, which is the second most common CFTR mutation. The G542X mutation is a nonsense mutation, and therefore categorized as a class I mutation [27]. Although the G542X mutation is more common in frequency than the G551D mutation (see below), it is not as widely discussed.

The third most common CF mutation is the G551D, which is found in the population at a frequency of about three percent. The G551D mutation causes a severe phenotype and is classified as a class III mutation. This mutation is caused by the glycine residue at position 551 being replaced by an aspartate residue. This amino acid replacement causes a large reduction in CFTR channel activity. Similar to the ΔF508 mutation, G551D is located within the NBD1. Little is currently known about the mechanism that causes decreased channel activity within G551D CFTR. The G551D CFTR exhibits normal synthesis, trafficking, and processing. The R domain in this mutation can also be phosphorylated normally, but the channel still remains inactive. It was discovered that the on rate of channel activation by cAMP is greatly reduced, while the closed time of the CFTR channel increases. The G551D CFTR mutant has no channel response to either ATP or ADP [28]. The G551D mutation has become popular recently due to a breakthrough treatment that I will discuss later in this paper.

These two mutations are just a sample of the numerous mutations responsible for CF. Currently, approximately 1500 different mutations in the CFTR have been discovered that are capable of causing the disease phenotype. Compared to the ΔF508 mutation, these other mutations are extremely rare and not widely discussed [8]. Currently, only 22 mutations have

been classified that occur with a frequency of over 0.1%. The other mutations are typically only seen in one or a few individuals [29].

Primary Complications with CF

Cystic Fibrosis causes multiple health complications. The primary effect of CF is within the airways and submucosal glands [29]. CFTR is expressed throughout the lungs where it helps to maintain the proper chloride levels. However, with mutant CFTR, the chloride levels are not able to be regulated properly. This leads to an increase in intracellular chloride levels and a decrease in extracellular chloride levels. The chloride imbalance causes excess movement of water into the airway epithelial cells, which leads to extreme dehydration of the mucus layer coating the airway surface. The resulting thick mucus is difficult to dislodge and obstructs proper airflow, which impairs lung function capabilities. This thickened mucus also acts as a perfect environment for bacterial infections. Due to the complications that can arise from thick mucus within the lungs, dehydration of the airway surface is typically considered the initiating event in lung disease within CF patients [30].

Within a healthy lung, the mucus is mostly composed of mucin glycoproteins that can either be secreted by the cell or tethered onto the membrane of the cell. Two of the most important mucins, MUC_5AC and MUC_5B, appear to be greatly reduced in CF patients. As mentioned earlier, the main cause for this thick mucus is the decreased available extracellular water. However, there also seems to be a secondary cause of viscous mucus within the lungs: an increase in anionic polyelectrolytes, including DNA made from the invading bacteria and DNA released from lysed inflammatory cells. Thick mucus is also partly a result of an increase in actin molecules. Regardless of what causes this viscous mucus, it eventually builds up in the CF

patients' airways and detaches from the cilia. This in turn inhibits the mucociliary transport, which is the main cause of lung morbidity and ultimately mortality in CF patients [30].

As mentioned before, the thick mucus lining the CF airways provides a perfect home for various bacterial infections. Shortly after birth, initial infection occurs from bacterial pathogens. The decreased function of the mucociliary transport prevents the clearing of the lungs. The antimicrobial peptides located in the airways become overwhelmed, allowing infections to take hold. Due to these infections, the CF airway shows a prolonged primary inflammatory response typical of a chronic infection. The manifestation of lung disease within CF patients is variable. Most individuals do not show symptoms while they are newborns, but signs of lung disease such as wheezing, coughing, and difficulty breathing can be seen in infants around six months of age. As an individual ages, coughing becomes a daily occurrence, usually accompanied by sputum. During later stages of the disease, it is not uncommon to find blood-streaked sputum [29].

A wide range of microorganisms are associated with pulmonary infections in CF patients. Infants and young children are most likely to be infected with *Staphylococcus aureus* and *Haemophilus influenzae*. Within older children and adults, *Pseudomonas aeruginosa* is more likely to be found within the airways [31]. *H. influenzae* is the most common microorganism found in the lower airways at age one. It is also non-typeable which means that the childhood immunization against *H. influenzae* type b has no affect in helping to prevent this infection. Currently, the role of this microorganism in the progressive pulmonary infection and inflammation in CF affected patients has not yet been clearly demonstrated, though it has been proven to be pathogenic in non-CF patients [29].

P. aeruginosa is the most significant microorganism associated with CF. Approximately 80 percent of CF patients will eventually be infected by this pathogen, resulting in clinical

deterioration. The source of *P. aeruginosa* is currently unknown, but the fact that most CF patients contain different genotypes of the bacteria suggests the infection comes from the environment [29]. Typically, chronic infection does not occur at first. Instead, there is a period of recurrent, intermittent colonization by the pathogen until the patient eventually develops a chronic infection. This normally occurs during the late twenties or early thirties in patients. In a chronic infection, there is a continuous growth of *P. aeruginosa* that leads to an increase in inflammation. This inflammation along with damage done by the bacteria itself is a major cause of damaged lung tissue and a decrease in lung function [32].

S. aureus is the most common pathogen found in CF children from ages 11 to 15 years. In the past ten years, *S. aureus* has gained a lot of attention due to the rise of methicillin resistant *S. aureus* (MRSA). Once MRSA has established itself within the CF airways, it is almost impossible to remove through antimicrobial therapy. Since 1960, approximately 80 percent of *S. aureus* has been resistant to penicillin, and within 2 years of the introduction of methicillin in 1959, methicillin resistant strains began to develop. Initially, MRSA was only seen in hospitals, but the prevalence of MRSA within CF patients has increased from 2 percent in 2001 to 22.6 percent in 2008 [33].

Following the development of MRSA, studies have investigated how MRSA affects CF patients in comparison to methicillin sensitive *S. aureus* (MSSA). It was found that CF patients infected with MRSA as the primary pathogen have lower lung function than those infected by MSSA. Patients with MRSA also show a higher rate of hospitalization and use of antibiotics [34]. MRSA CF patients also exhibit a faster decline in lung function than those without MRSA. Currently, treatment for MRSA has no guidelines but typically includes several different antibiotics used to help control the MRSA infection. There have also been some rare cases of

eradication of MRSA in CF patients which is encouraging, in that MRSA might be able to be eradicated by the use of systematic antibiotic therapy [33].

Another resilient pathogen has also made its way into the CF community within recent years. This devastating bacterium is the *Burkholderia cepacia* complex (BCC). It can be found in multiple environments, including agricultural crops and aqueous solutions such as detergent solutions and intravenous fluids commonly found in hospitals. BCC was not a prominent pathogen within CF until recently, because patients had a shorter life expectancy. Now that patients with CF are living longer, BCC has been able to take hold and can be found in about ten percent of adult CF patients. A pulmonary infection of BCC can persist for months or even years. BCC can lead to severe disease or death within as little as six months. However, every individual reacts to BCC differently, with some patients not even experiencing a decline in clinical status after infection [35].

Managing CF patients infected with BCC leads to a serious problem because BCC is resistant to most antimicrobials and no new antibiotics have been produced that can kill this pathogen. BCC might be susceptible to some antimicrobials, but once it encounters a new antibiotic, it quickly develops a resistance. This leaves doctors with the problem of trying to treat a patient with a pathogen that is resistant to all known antimicrobials. There have been some reports of successful treatment of BCC in CF patients, but these reports are rare. The fact that CF individuals can become infected with BCC from the environment or from other fellow patients has led to great anxiety amongst the CF community. Infection control procedures were soon put in place to help prevent the spread of BCC. CF summer camps were shut down and patients with BCC were segregated from all other CF individuals. BCC infected individuals are no longer allowed to go to any CF gatherings such as fundraisers, and CF patients in general are told to

keep their distance from each other in order to help prevent any unintentional spreading of BCC [35].

Another major complication found with CF is within the digestive system. Similar to the lungs, patients with CF have a coating of thick mucus within their intestines. Defective CFTR proteins within the digestive system causes impaired intestinal fluid secretion because the water moves into cells that are filled with chloride that cannot escape. This in turn leads to constipation and mucus filled stool. As mentioned earlier, constipation caused by CF helps to prevent death by Cholera, which is a leading theory for why CF is so prevalent [36]. The thick mucus in the digestive tract can also block pancreatic secretion of digestive enzymes that aid in the digestion and absorption of food. A blocked pancreas in CF patients can lead to pancreas insufficiency, malabsorption of fats and proteins, and a possible deficiency in fat-soluble vitamins [37].

A third major complication within CF adults is infertility. Approximately 98 percent of CF males are infertile. This infertility is caused by various different problems within the male reproductive system. Ultimately, almost all CF males are azoospermic, meaning that they do not have a measurable amount of sperm within their semen. Male CF patients typically have bilateral absence of the vans deferens. The body and tail of the epididymides are deformed or absent. The testes may or may not be reduced in size, and the seminal vesicles typically contain various abnormalities. It has been proposed that these abnormalities are caused by a failure of differentiation during the first trimester [38].

Female CF patients on the other hand do not have any reproductive system abnormalities. However, women with CF sometimes have a hard time getting pregnant due to the blockage of the cervix by mucus. Patients who decide to have a child must carefully plan out their pregnancy. Women must weigh issues such as a loss of lung function during pregnancy and the toxicity of

certain drugs to the fetus. Typically women regain their lost lung function after giving birth, but some women fail to regain their lung function and die as a result. Therefore, it is imperative that CF patients talk to their doctors and make the best decision for their individual case as to whether they should become pregnant [38].

Secondary Complications with CF

Along with the primary complications caused by CF, many patients experience secondary complications or comorbidities. One of the most severe secondary complications found with CF is diabetes. CF diabetes is a separate entity from type 1 and type 2 diabetes and is known as cystic fibrosis-related diabetes (CFRD). CFRD is normally found within CF patients who have more severe forms of the disease, and is associated with an insufficient pancreatic exocrine function. CFRD leads to an insulin deficient state typically without ketoacidosis. The traditional model for the onset of CFRD is the "collateral damage" model. According to this model, thick mucus resulting from defective CFTR proteins leads to blockage and damage of the pancreas. Soon progressive fibrosis and fatty infiltration takes place, leading to the destruction of the hormone producing areas of the pancreas. Therefore, less insulin is produced and released into the blood stream [39].

As healthy individuals age, it is normal for β-cell function to decline, leading to decreased insulin production. This natural decline along with β-cell abnormalities associated with CF, has led to an increase in CFRD as CF patients survive to an older age. CF patients with CFRD have a six fold greater mortality rate than those patients without diabetes. CFRD is also associated with lower lung function in CF patients. The decline in lung function can begin several years before the patient is diagnosed with CFRD. Another early sign of CFRD is a decrease in BMI. Although CFRD is a severe complication associated with CF, improvements in

survival have been seen with early diagnosis and treatment. Therefore, now that CFRD is more common and better understood, doctors can screen their patients to catch the onset of diabetes as early as possible, greatly increasing the patient's chance of survival [39].

Another secondary complication associated with CF is osteoporosis. However, in order to prevent any confusion with osteoporosis found in patients without CF, CF osteoporosis will be referred to as bone disease. Low bone mineral density (BMD) was first noted in CF patients in 1979 [40]. Since low BMD is already common in the elderly population, the fact that CF patients are living longer has led to an increasing importance of understanding causes behind bone disease in CF patients. Within the CF population, fractures occurring due to bone disease typically are located within the vertebrae. It has also been noted that fracture incidents are more frequent in the female CF population. These fractures not only cause pain, but they can also prevent effective treatment of the lung disease associated with CF. Many treatments for CF lung disease induce coughing and methods to break up mucus within the lungs, and a fracture within the spinal cord would make these types of treatments impossible [41].

Currently, the mechanisms behind bone disease within CF patients are not completely understood. However, many risk factors for low BMD have been identified. The first risk factor is insufficient pancreatic activity. As mentioned earlier, this leads to malabsorption of nutrients therefore leading to malnutrition and a lower BMI. Both poor nutrition and low BMI are known to contribute to lower BMD values. Another issue with poor nutrition is decreased calcium absorption that is not reversed through the use of supplemental digestive enzymes. The second risk factor for bone disease in CF patients is vitamin D and vitamin K deficiencies. Both of these vitamins are important for the maintenance of bone structure. Due to poor pancreatic activity, these vitamins are not absorbed well, plus patients with CF typically do not take part in outdoor

physical activities leading to an even greater vitamin D deficiency. A third factor is limited physical activity within CF patients. Bone mass increases with exercise due to force placed on the bone. However, since CF patients are normally not active, their bones do not have the proper pressure to strengthen. Bone disease in CF patients can also be caused by recurrent infections that lead to the reabsorption of bone. Also, glucocorticoids are typically used for CF patients to improve lung function, but these medications are known to decrease BMD. Finally, it has recently been discovered that the defective CFTR might lead to the dysregulation of genes important for bone formation [40].

CFRD and bone disease are just two of the many different secondary complications associated with CF. Another common complication is the development of pancreatitis. As mentioned earlier, mucus plugs the pancreas and leads to a decline in its function. If the pancreas becomes completely blocked, this can lead to pancreatitis. It has also been found that as CF patients are reaching older ages, their risk for gastrointestinal and pancreatic cancers is higher than average [42]. Other secondary complications can include pain such as chest pain and headaches. This pain not only reduces the patient's quality of life, it also might interfere with a patient's treatments. CF can also lead to complex social interactions where individuals struggle to explain CF to colleagues and friends. Serious psychiatric disorders are also associated with CF. Rates for both major depression and suicide are higher for adult individuals suffering from CF. This list of complications is still just a short overview of the myriad of complications that can be brought on by CF [43].

Current Treatments

Many treatments for CF have been discovered over the years. These treatments typically treat the symptoms of CF and differ from patient to patient. One of the most common treatments

today is hypertonic saline which is inhaled by patients through breathing treatments. The use of hypertonic saline in adults with CF has been proven to improve mucociliary clearance. Hypertonic saline works through two separate mechanisms. First, it targets the dehydration effect of CF within the lungs. By breathing in saline solution, water is drawn out of the cells within the lungs helping to loosen the thick mucus caused by defective CFTR proteins. Second, hypertonic saline induces coughing within patients. These coughing spells improve mucociliary clearance by creating a sheer force that promotes the removal of mucus within the airways. The increase in mucociliary clearance leads to better lung function within CF patients [30].

Another common inhaled treatment for CF is pulmozyme or dornase alfa. Pulmozyme was approved in 1994 for use in the treatment of CF [44]. This treatment contains an enzyme known as deoxyribonuclease I (DNase I). DNase I cleaves DNA, which can reduce the viscosity of mucus within CF patients. Due to chronic inflammation within CF lungs, there is an excess of cellular debris. This debris in turn increases the viscosity of the surrounding mucus. Therefore, pulmozyme works to reduce viscosity by digesting the extracellular DNA accumulated within the lungs. Similar to hypertonic saline, the loosening of mucus by pulmozyme allows for better clearance of the lungs. This leads to an increase in lung function and a decrease in respiratory exacerbations. Pulmozyme is also well tolerated in patients, making it an ideal treatment for those with CF [45].

Other treatments for CF include different types of chest physiotherapy. The oldest of these methods is conventional chest physiotherapy, which was introduced in the 1950s. This treatment involves pounding on the back of the patient in a head-down position which helps to slow the progression of declining respiratory function. Currently this treatment is only used in children due to side effects such as abnormal cardiac rhythms in adults. In the 1970s, another

chest physiotherapy method was introduced called the PEP mask or more commonly the flutter. The flutter requires the patient to exhale through a mouthpiece that generates resistance. This resistance creates positive pressure within the airways, forcing air into the more distal lung regions in order to break up thick mucus secretions. The PEP mask has been found to be just as effective or more effective than the conventional chest physiotherapy method [46].

One of the most effective chest physiotherapy methods is high-frequency chest compression. This has been shown to increase mucociliary clearance by increasing expiration and creating forces leading to coughing. Eventually, a device was created called the Vest Airway Clearance System. The Vest works by applying high-frequency oscillations to the patient's chest via an air-pulse-generating compressor. These oscillations help to move mucus secretions in the small airways into larger airways where they are more easily removed by coughing. This treatment is normally well tolerated, but in some patients with more advanced lung disease, it can cause pain and discomfort. Overall, the Vest is a good alternative for the conventional chest physiotherapy method and has been shown to decrease the rate of decline in pulmonary function over a long period of use [46].

Another main focus of CF treatment is controlling pulmonary infections. During early lung disease within CF patients, doctors typically prescribe antibiotics to help delay the chronic colonization of pathogens. Once pathogens have colonized, chronic maintenance antibiotics are given to patients to help slow the decline in pulmonary function. During times of increased pulmonary symptoms, patients are typically prescribed a more intense antibiotic regimen. Typically, these intense treatments are carried out through intravenous methods whether they be at a hospital or at home. Less intense antibiotic regimens are typically inhaled during a patient's regular treatment times. Since there are a wide variety of bacterial infections that can take hold in

CF patients' lungs, there are also a large amount of antibiotics. Antibiotic treatments are individualized for each patient. There is no clear cut treatment plan for infections within CF patients [29].

In order to treat pancreatic insufficiency in CF patients, individuals are typically advised to alter their diet and consume supplemental pancreatic enzymes. To help prevent stomach issues, patients are advised to reduce their fat intake. It is also advised to reduce the intake of hard to digest foods. Along with a modified diet, patients take pancreatic enzymes to aid the digestion of fat and the absorption of some nutrients. Since enzymes in healthy individuals are typically released parallel to eating a meal, supplemental enzymes usually are taken at the same time as a snack or meal. These pancreatic enzymes not only help in digestion and absorption of nutrients, they also help in reducing stool frequency and improving stool consistency in CF patients. Execution of this therapy should be based on an individual level depending on each patient's nutritional status and pancreatic damage [47].

The above treatments are just a few of a wide range of options for CF patients. Along with these treatments, doctors typically advise patients to follow an exercise regimen. Exercise has been proven to have a therapeutic effect on CF individuals [48]. Another possible treatment for CF is a lung transplant. This is a rescue therapy used in both adults and children who have terminal lung disease. The first lung transplantation for a CF patient occurred in 1983. There are many complications that occur during a lung transplantation, but if all goes well, a transplant can have dramatic results on a CF patient [49]. All of these treatments depend on each individual patient, and only a doctor and the patient can decide what treatment route is the best fit for their unique situation.

Latest Breakthrough Therapies

The latest breakthrough for the treatment of CF is Vx-770 more commonly known as Kalydeco. Vx-770 was released in 2012 and is the first FDA-approved drug that directly affects the CFTR [50]. In the trial stages for Vx-770, it was found to have the greatest effect on CFTR proteins with the G551D mutation. A study was carried out that tested the safety and effectiveness in CF patients with at least one G551D allele. In the study, it was found that patients given Vx-770 showed significant improvements in lung function. CFTR function in nasal epithelial cells and sweat glands also showed improvement. During the study, there were a few adverse effects noted, but these were uncommon and relatively minor. It was determined that Vx-770 could be a viable therapeutic treatment for CF patients with the G551D mutation [51]. Although Vx-770 has already been approved, the exact mechanism by which the therapy works is not understood. Vx-770 is a CFTR potentiator and increases the open time of the CFTR channel. The binding site of Vx-770 is also unknown at this time. Unfortunately, this therapeutic treatment is only applicable to those with the G551D mutation, which only accounts for about three percent of the CF population. It has also been shown that Vx-770 is not a cure for G551D patients, but it offers a significant increase in patients' health and provides hope for all those with or involved with CF [50].

Other therapies are now making their way through the trial phases for the treatment of patients with the ΔF508 mutation. One of these therapies is Vx-809 combined with Vx-770. This therapy recently passed the second phase of study and is now in the works of a phase three study. Vx-809 is known as a CFTR corrector, and it helps the CFTR protein reach the cell membrane. The Vx-770 then works as a potentiator to help keep the CFTR channel open for longer periods of time. In the phase two trial, there was a significant increase in lung function for patients taking

the combination of Vx-809 and Vx-770. The combination of treatments also appears to be well tolerated with patients, with the most severe side effects being pulmonary in nature. Soon the phase three trial will be conducted for Vx-809 and Vx-770, providing hope for individuals with the most common CF mutation [52].

Another breakthrough therapy undergoing trials for the ΔF508 mutation is the combination of Vx-661 and Vx-770. This combination therapy just recently finished its phase two study. Like Vx-809, Vx-661 is a CFTR corrector used to help move the ΔF508 mutated CFTR to the apical membrane. This combination therapy also seems to be well tolerated by patients, with only some mild to moderate pulmonary side effects. The study showed significant decreases in sweat chloride production, which is unusually high in patients with CF. With high enough doses, Vx-661 and Vx-770 together led to a significant increase in lung function. Along with Vx-809 and Vx-661, there is still another potential corrector for CFTR being tested. However, of these three correctors, Vx-809 is currently the leader, providing the greatest hope for a radical treatment for those with the ΔF508 mutation [53].

Currently, there is another potential therapy moving through the trial stages called ataluren. This therapy is effective for patients who have a nonsense mutation in their CFTR gene. Ataluren is a drug that allows for ribosomal readthrough of mRNA molecules that contain premature stop codons. It was determined that ataluren leads to the proper translation of a CFTR protein. Although the trial period was short, there were trends towards higher lung function and a decrease in coughing. These results prove that there could be a clinical benefit of ataluren in individuals with a nonsense mutation in their CFTR gene. Further research is needed to access the improvement in lung function before this drug will be ready for release to the general CF

population. Although it has not been released yet, this is another possible therapy for individuals suffering from CF [54].

Conclusion

Cystic Fibrosis is a terrible disease that still has no cure. However, progress since the discovery of CF in 1938 has come in leaps and bounds. CF patients have gone from not living beyond childhood to a life expectancy well into adulthood. Scientists have a better understanding of the causes of CF and therefore can now focus more attention on developing treatments. Over the past few years, new radical prospective therapies have been making their way through trials. The release of Kalydeco was a monumental moment for the CF community. Hopefully, Vx-809 soon will be released along with Vx-770 to greatly increase the quality and length of life for those with the ΔF508 mutation. No matter what happens in the future, there is no doubt that the outlook on CF has changed dramatically over the years. At the rate that discoveries are being made in the CF community, I have no doubt that within the near future, CF patients will be able to live healthy, normal lives with the help of amazing new therapies.

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