CYSTIC FIBROSIS:
A DRIVE FOR ACQUIRING KNOWLEDGE

By

KATHERINE VIRGINIA BULLOCK

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Approved by:

Dr. Douglas Keen
Department of Physiology
Abstract
Cystic fibrosis (CF) detrimentally affects the pulmonary and digestive system of patients and only a few years ago had a life expectancy of a mere 40 years. However, due to strides made in CF research the life expectancy projection is now 58 years. Cystic fibrosis transmembrane conductance regulator (CFTR), an anion channel located on the apical membrane of epithelia, is directly responsible for the translocation of chloride and indirectly responsible for the paracellular translocation of water. A mutation in CFTR causes systemic hyperosmotic epithelial secretions. My thesis focuses on existing knowledge about CF with emphasis on the basic defect of the disease and the current standard of care. Recent and noteworthy papers were collected and synthesized. Current treatments were examined for specificity and success in reducing symptoms. CF research is making great advancements in individualized treatment of this disease which has vast variation from patient to patient due to the vast number of mutations that can affect CFTR. CF research is an excellent example of how targeting specific mechanisms of a disease can decrease morbidity in CF patients. Having comprehensive knowledge may help in the understanding necessary to continue focused basic research and alleviate the health problems associated with CF.
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Introduction

“There is not a true life expectancy for this disease because there are so many varying factors that contribute, but on average, people with CF are living into their 30's and 40's. That being said, there are those who live much longer than that... my goal is to be one of those.”

This quote was written by Kayla English, a blogger with cystic fibrosis (CF), and describes the drive for not only survival but happiness that is apparent in many cystic fibrosis patients (9). Cystic fibrosis is a multisystem genetic disease characterized by lung disease and pancreatic insufficiency. It is the most common genetic disease among Caucasians with a live birth rate of 1 in 3,200 in the United States. This genetic mutation results in a nonfunctioning or absent protein responsible for chloride movement in epithelial cells. The absence of this process results in thick mucus secretions that causes pancreatic insufficiency and an increased susceptibility to lung infections. This sounds discouraging and overwhelming; however, great advances have been made in understanding and treating CF. The goal of this thesis is to summarize what knowledge has been acquired about CF and the progress that’s been made towards individualized treatments.

While writing this thesis, I read many scientific articles that dispassionately describe what cystic fibrosis is and how we treat it. However, it is the stories of dedication and perseverance that have driven me forward. They are the reason I chose this disease in the first place and why I’ve compiled this summary of the disease and the current diagnosis and treatment options.
Cellular & Molecular Basis of Cystic Fibrosis

Genetics
Cystic fibrosis (CF) occurs in patients with an autosomal recessive gene, meaning that he or she inherited two recessive genes, one from each parent (5). Because CF used to result in mortality at such a young age and some symptoms may include infertility, it is extremely rare for patients to reproduce (18). Therefore, cystic fibrosis is most often passed from two parents without CF, but who are carriers for the recessive gene linked to CF. In Caucasians, the population with the highest prevalence of CF, the carrier rate is 1 in 25 people (4). The CF mutations may either be homozygous or compounded heterozygous, meaning that there must be a mutation present in each copy of the gene, but the mutation does not necessarily have to be the same. There are thousands of mutations linked to cystic fibrosis and therefore this phenomenon is not uncommon in cystic fibrosis patients (31). This means that cystic fibrosis patients present with a relatively large variety of phenotypes (5).

The DNA present in each of our cells contains all of the genetic information that allows for the creation of proteins that are responsible for all bodily functions. DNA is organized into chromosomes, which are made of genes responsible for individual proteins. Using a technique known as molecular cloning, Bat-sheva et. al. determined there is a single CF locus that exists on human chromosome 7 (16). The CF gene is about 230,000 base pairs long with 27 exons (41). Genetic information is transcribed into a messenger RNA molecule (mRNA), which can then go through splicing modifications that act to remove unnecessary information. The mRNA functions as the directions for the formation of the proteins. Each protein has a specific function contributing to complex bodily functions. It is known that protein malformations can be caused by issues at one (or more) of many stages in protein formation listed above; however, according to Riordan et. al., the crucial error that leads to the symptoms in CF patients is isolated to the
genetic information (25). This means that the error occurs from the deletion of one or more base pairs and the subsequent protein malformation is consistent in all types of epithelial cells of the patient. This channel is responsible for the translocation of negatively charged chloride across the plasma membrane in epithelial cells. It has been shown that there are thousands of mutations on the CF gene that can lead to CF symptoms (21). Genomic DNA samples of CF patients and their parents have been used to determine the frequency of the various types of mutations (8).

**Classes of Mutations**

Cystic Fibrosis mutations are placed into classes based on the effects of the mutation on the biogenesis of the cystic fibrosis transmembrane conductance regulator (CFTR) channel (32). Class I CFTR mutations are characterized by a defective production of the CFTR protein, which leads to no function, nor any expression of the CFTR protein at the membrane. Class II CFTR mutations result in impaired processing of the protein. These mutations result in nonfunctioning proteins that are not expressed at the plasma membrane; the ΔF508 mutation, to be discussed later, is an example of this type of mutation. Next, class III mutations result in defective regulation of CFTR. This also means the patient has no functioning CFTR channel, but these channels are expressed in the membrane. Class IV mutations are characterized by the conductance of the channel being defective. This mutation is the first, thus far, that results in functioning CFTR; however, their function is significantly reduced. Lastly, class V mutations produce CFTR that is translocated to the membrane but smaller amounts of the protein are produced (32). Classes I-III are considered severe mutations and classes IV and V are considered mild based on the phenotypes and symptoms that occur in the presence of the corresponding mutations.
Biogenesis & Mutations

The presence of certain mutations affects the biogenesis of CFTR. However, the normal biogenesis of CFTR starts with the translation of the CFTR protein in the rough endoplasmic reticulum (ER). Simultaneously a glycan (sugar molecule) is attached to a nitrogen atom of the protein in a process called N-linked glycosylation. Also during translation the protein is folded and refolded in a process that maximizes the bioenergetics of the system. ER-associated degradation (ERAD) is constantly interacting with the protein, checking for deleterious errors. This quality control process is highly effective and accounts for about 80% of the degradation of abnormal CFTR proteins (32). If a protein is abnormal, like in the case of a ΔF508 mutation, chaperone complexes lead to the ubiquination and decomposition of the abnormal CFTR (6). Next, CFTR is transported to the Golgi complex via intracellular translocation pathways. Here, CFTR is prepared for translocation to the plasma membrane. Further glycosylation occurs in the
Golgi complex. The transportation of CFTR to the plasma membrane is modulated by PDZ-domain-containing proteins (32). The healthy biogenesis of CFTR will lead to functional chloride channels, but if there is a mutation present some step of the biogenesis may be disrupted.

Based on the putative frequency of the mutations and the results of the various types of mutations, I have chosen to focus on four common mutations that also have four unique mechanisms that all lead to CF symptoms and cover classes I-IV. The four mechanisms describe at what point in the process of the protein formation and activation the error affects the function of the protein. The genetic mutation will prevent the CFTR channel from forming properly, prevent the formed channel from being delivered to the membrane, prevent the channel from functioning within the plasma membrane or prevent full function of the CFTR channel in the plasma membrane.

The first type, a class I mutation, is the R553X nonsense mutation. It has a high prevalence in Hispanic populations and accounts for about 1% of CF patients (41). This nonsense mutation indicates that there is a single base pair substitution at the 553rd amino acid, where normally an arginine resides (32). In the case of this mutation, a premature stop codon is coded and the protein is truncated before the complete formation of the protein. The premature stop codon terminates the translation of the protein from mRNA, therefore forming an incomplete protein that cannot fold properly or function as a normal protein. Mechanisms within the cell recognize this protein as insufficient and destroy it before it can be transported to the cell membrane. Due to this, there is a defective production of CFTR and no CFTR channels at the apical membrane of the epithelial cells.
Another type of mutation is a class II mutation called the ΔF508 mutation and is the most prevalent mutation, occurring in about 66% in CF patients (41). This mutation leads to the deletion of three nucleotides that make up one codon. The deletion of this codon results in a missing phenylalanine in position 508 in the first nucleotide-binding domain on the gene. The missing phenylalanine prevents the protein from folding properly because the subsequent charged residue has taken the place of the phenylalanine, which has a nonpolar residue. Therefore, the charged residue does not interact with the other hydrophobic residues. These lack of interactions result in an inability of the protein to fold properly, which traps the protein in the endoplasmic reticulum and prevents any further processing. Similar to the protein that results from the R553X mutation, the protein does not get delivered to the membrane and therefore those cells are completely missing the CFTR channel protein, preventing the conductance of chloride. In the case of both the R553X and ΔF508 mutations, the misfolded proteins are recognized first by the endoplasmic reticulum quality control (ERQC) mechanisms. These proteins do not pass the criteria of the ERQC and therefore are degraded by the endoplasmic reticulum associated degradation (ERAD) pathway. This pathway first recognizes the protein as misfolded in the endoplasmic reticulum, using cytoplasmic and luminal chaperones. Next, through a process called retrotranslocation, translocation machinery or E3 ligases transport the erroneous protein into the cytoplasm. Next, E3 ubiquitin ligases polyubiquitylates the protein, thus labeling it for degradation (7). De-ubiquitylating enzymes recognize the label and guide the protein through the catalytic region of a proteasome. This decomposes the CFTR protein into polypeptide fragments, ready for reuse (40).

The G551D mutation accounts for the third most common CF mutation in the world and affects approximately 4 to 5% of CF patients (23). This is a class III mutation and therefore
results in a translocated, but non-functioning protein (41). The G551D is a missense mutation; a missense mutation is caused by an error in one nucleotide and results in the translation of a serine instead of a glycine at the 551st amino acid position on the protein (32). This is in the first nucleotide-binding site of the protein. This amino acid replacement leads to the insertion of polar side chains into the ATP-binding site (43). This abnormal protein will significantly decrease the channel functionality of the CFTR protein such that it is essentially nonfunctioning. In this case, the CTFR protein is present in the plasma membrane but chloride translocation is significantly diminished or stopped completely.

The last mutation type discussed here is the missense R117H mutation. This occurs when an arginine is replaced by a histidine at the 117th residue (34). This in considered a class IV mutation due to its decreased conductance of anions through CFTR. This mutation is completely transcribed and makes it to the plasma membrane of the epithelial cells, like the G551D mutation. However, this mutation is different because it is somewhat functional. There is conductance of chloride across through CFTR, but it is abnormal. This abnormality is due to an effect from the mutation on the second and sixth membrane-spanning domain. Therefore, the regulation of the activity of this channel is normal, but it’s been found that there is a decrease in the movement of chloride across the channel or a decrease in conductance (34).

Though there are more than a thousand mutations that result in cystic fibrosis, the examination of the types of mutations has inspired individualization of treatment and research for cures (27). By examining the genetic mutations, one can predict the changes in the structure of the CFTR and therefore how its functionality is affected. Though these mutation lead to similar symptoms, the identification of precise locations in the pathway will lead to treatments that terminate or repair the occurring errors.
The CFTR Protein

CFTR is located in epithelial cells including the lungs, skin, pancreas, liver and the digestive tract. Specifically, CFTR resides in the apical side of the membrane of the epithelial cells (32) and is responsible for the movement of chloride and other anions out of epithelial cells and into the lumen of various organs. The energy stored in ATP allows for a conformational change in CFTR that regulates the passage of the ions down their electrochemical gradient. The decrease in chloride transport is coupled with an accelerated sodium absorption by the epithelial cells. Water also follows these ions across the cell layer paracellularly. This movement of sodium and therefore water away from the mucosal membrane is what leads to the thick mucous characteristic in CF patients (2). Therefore, this channel also regulates the transport of salt, ions and the flow of fluids across the epithelium. CFTR is termed an ABC transporter, because it is an ATP-dependent cassette transporter (13) and has 5 crucial domains: one regulatory domain, two nucleotide-binding domains (NBD) and two membrane-spanning domains (MSD) (35).

![Proposed model of CFTR](image)

**Figure 2.** Proposed model of CFTR. MSD: membrane-spanning domain; NBD: nucleotide-binding domain; R: regulatory domain; PKA: cAMP-dependent protein kinase (35).
The mechanism of opening the normal CFTR channel begins with the phosphorylation of the regulatory domain by protein kinase A (PKA) at various serine residues. The regulatory domain controls the activity of the channel and therefore can be phosphorylated by molecules other than PKA, which leads to stimulatory or inhibitory regulation of the channel (13). Overall, the control of the activity of the channel is regulated by kinase and phosphatase activity within the cell. After phosphorylation of the regulatory domain, ATP binds with the first nucleotide-binding domain. This ATP molecule then forms a dimer with the first and second nucleotide-binding domains at which point the ATP molecule is then hydrolyzed. This allows for the activation of the chloride channel. Lastly, protein phosphatases dephosphorylate the regulatory domain and CFTR returns to a quiescent state (13). In a healthy individual, the building, translocation and proper gating of CFTR will result in very important mucus secretion throughout the body. However, in patients with CFTR gene mutations these process are altered in a way that hinders the healthy mucus secretions.

The first two mutations discussed, the R553X and ΔF508 mutations do not produce functional proteins due to a lack of production and impaired processing, respectively. However, the G551D and R117H mutations produce proteins that make it to the membrane, but have a lower functionality. In the case of the G551D mutation, glycine, a small nonpolar amino acid, is replaced with a serine, a larger polar amino acid, at the 551st amino acid on the CFTR protein. This is the location of the first nucleotide-binding site and is called a gating mutation because it affects the regulation of the protein (32). The location of this mutation is particularly important because it occurs at the interface between the first and second nucleotide binding sites. This severely inhibits the ATP-dependent gating and decreases the probability that the channel will open by 100-fold when compared to a healthy CFTR (32). This is due to a decrease in the
frequency of the opening of the channels (35). In this case, there is nearly no movement of chloride through this channel.

In the case of the R117H mutation, arginine has replaced histidine at the 117th amino acid (32) on the outside of the second membrane-spanning segment (35). Sheppard and Welsh determined that the R117H mutated protein was still activated by cAMP, but that the conductance was 30% less than the wild-type CFTR (35). It is believed that the pH outside of the cell affects the conductance of these channels as well. This is because in an experiment with Sheppard et. al. (1993) the external pH was greater than 8.8, the conductance of the channel significantly dropped, but this was reversed when the pH was brought back to a physiological standard (34). This suggests that the location of the amino acid replacement is likely on the external portion of the protein. Because there is some conductance of chloride across the channel the phenotypic differences between the R117H CFTR and the wild-type CFTR are milder than in any of the other mutations discussed. This is true for most class IV and V mutations.
**Diagnostics**
The diagnosis of cystic fibrosis is a systemic two tier analysis of test results. The testing may begin before or after birth. Family history and early symptoms may also initiate the screening process for cystic fibrosis patients and their families. Prenatally the parents may chose to genetically test the fetus and this may be due to family history along with a variety of other reasons. On the other hand, newborn screening (NBS) is required for all newborns in the United States. Figure 1 outlines the screening process.

**Figure 3.** Screening for Cystic Fibrosis. The general protocol for CF screening in the United States. There are variations such as different screening techniques or a change in order depending on the particular patient.

**Prenatal Screening**
Prenatal screening is the first opportunity for the diagnosis of cystic fibrosis. Genetic screening is available and recommended for all pregnant women in the United States (3). The genetic test identifies 23 different mutations of the CFTR gene. The genetic testing can involve identifying a fetus and/or parents with one or two mutations of the CFTR gene. If parents discover that one or
both are carriers of CFTR gene mutations then they are provided educational material about the possibility of having a child with cystic fibrosis. Because there are thousands of mutations and mutation combinations that can cause a wide range in the severity of symptoms, at this stage there is still a large amount of uncertainty. Some patients with a CFTR mutation are not widely affected, but in other cases a patient may have significant morbidity (10).

While this knowledge allows doctors and family members to act promptly in order to slow any symptoms of cystic fibrosis, there are downsides to prenatal genetic screening. For those carrier couples, this discovery could lead to changes in reproductive plans. This could lead to the termination of a pregnancy, if prenatal testing is completed, which has vast ethical implications. Parental responses may lead to depression or other adverse effects. While these possibilities should be carefully considered, one survey of parents of children with CF and adult CF patients found that 80% supported preconception carrier screening for CF gene mutations (14).

**Newborn Screening (NBS)**

In the United States, there is a standard newborn screening for congenital genetic abnormalities (3). This early detection has been shown to improve the outcomes for patients and families due to earlier onset of treatment and preventative measures (42). Furthermore, patients detected via newborn screening techniques had improved scores on the Schwachman-Kulczycki (SK) score. The SK score is the first and most widely used test used to determine the severity of cystic fibrosis symptoms. It is a useful tool for determining the progression or changes of the disease in cystic fibrosis patients (36).

The disadvantages to NBS are similar to those of prenatal screening. This includes depression and anxiety in parents possibly due to a misunderstanding of a genetic carrier meaning. This depression and anxiety can lead to issues with parent-child bonding. Another
disadvantage is that these patients may be unnecessarily given medication in the cases of diagnostic errors (30). Overall, however, the newborn screening have been found to lead to an improvement in patient outcomes (3).

**Typical Screening through IRT and DNA testing**

The newborn screening process involves two tiers. In the first tier, the functionality of immunoreactive trypsinogen (IRT) is measured. IRT is a pancreatic exocrine biomarker that is typically elevated in infants with partial or nonfunctional CFTR. Immunoreactive trypsinogen has two isoforms, IRT-1 and IRT-2, cationic and anionic, respectively (37). Current techniques that test for both of these isoforms requires blood spots followed by assaying the samples for the presence of IRT (24). The threshold for IRT levels indicating the necessity for further testing varies per state, country and tier two protocol, but is generally within the 94-98th percentiles or greater than or equal to 65 µg/L of IRT (3). These values are debated because of the inconsistency in results due to seasonal and reagent variations (17). Historically, IRT testing has high rates of false positives, up to 85.9% in some studies (26). This level requires further testing, the second tier of diagnostics. Some cases of CF do not present with elevated IRT due to meconium ileus, a bowl obstruction due to thicker and stickier ileum (3). This is indicative of CF and therefore prompts further testing.

The next tier of testing is either a repeat the IRT test or complete a DNA test. The NBS genetic tests for CF are very similar to the prenatal genetic screening described above. Additionally, in either protocol, a sweat chloride (SC) test is also completed in order to confirm the results. SC tests are well established as an assessment for CFTR abnormality and the diagnosis of CF. Since CFTR is involved in sodium reabsorption, when it is not fully functioning, sodium is not reabsorbed and the skin sweat has excess sodium. Many mothers will first notice something when they kiss their baby and he/she tastes salty. This test stimulates
sweating and measures the sodium concentration. It is measured against standards to determine if sodium levels are elevated. These observations can be somewhat subjective, as the “standards” vary somewhat dependent on the state or country. The SC content also varies by age and therefore there are different standards for those under 6 months. While this test is a good tool for diagnosis of CF, there are multiple conditions under which a patient might get a false positive SC test, which include: anorexia nervosa, autonomic dysfuction, Mauriac’s syndrome, nephrogenic diabetes insipidus and psychosocial failure to thrive (3).

Fecal Elastase
While these are the most commonly used and well known diagnostic tools, there are a few others that can help to confirm a CF prognosis. The first is the measurement of fecal elastase (FE). A positive result from the FE test would suggest pancreatic dysfunction. A common result of non-functioning CFTR proteins. It is important to note with this test that the fecal elastase levels vary during the first year of life, and a follow up test should be completed after 1 year of age to decrease the chances of a false positive that leads to an incorrect diagnosis.

Nasal Potential Difference
Another test, is the nasal potential difference (NPD) test. This test is often used in clinical research to measure restored CFTR function, but can be used for diagnostics as well. This process involves placing electrodes across nasal mucosa which measure the potential difference as various solutions at set concentrations are perfused. Since, CFTR is responsible for chloride (an anion) transport, the electrodes are able to pick up on differences with high sensitivity (28). The benefits of this technique is the sensitivity to changes in potential difference of the nasal mucosa, however, there are yet to be standardized procedures. This means that the results may be interpreted differently or may not be comparable due to difference is protocol.
**Treatments**

There are two main categories for cystic fibrosis treatment; treatments that target the initial defect that is causing the malfunctioning protein and treatments that target symptom management. In this paper, we delve into the basic defects, the treatment of respiratory symptoms and the nutrition requirements. Both, the basic defect and symptom treatments, are very important for the progress in CF treatment research, as one helps decrease the symptoms a patient has and the other helps manage what symptoms they do have. Recently, there has been an increase in research producing results suggesting individualized treatments for the initial defect. This is crucial because unlike some genetic diseases, there are many combinations of mutations that lead to CF through unique mechanisms. The development of these targeted treatments is showing promise for not only CF treatments, but also other genetic diseases.

**Basic Defect Treatments**

There are four major therapies that attempt to correct or make up for the CFTR mutations: CFTR potentiators, CFTR correctors, gene therapy and translational readthrough. These function through different mechanisms to increase the correct cellular processing and delivery of the CFTR protein. The goal is to have treatments for all classes of CF mutations, but due to the large variation between classes, most treatments are especially beneficial for a single or couple classes.

**CFTR Potentiators**

One key treatment that works to remedy the basic defect of CFTR is CFTR potentiators. This treatment acts to activate pre-existing CFTR channels in the cell membrane. This is particularly useful for Class III mutations, such as the G551D mutation discussed above, but may be used for Classes I, II and IV (29). The mechanism of CFTR potentiators is to increase the likelihood of cAMP dependent channels opening by decoupling with ATP hydrolysis (38). This
therapy potentiates CFTR function by increasing the open time of healthy and mutant CFTR proteins already active in the cell membrane and is hypothesized to be because of the hydrolysis of more than one ATP molecule per one opening/closing event (15). There are still unknowns about the mechanism of specific CFTR potentiators such as Vx-770 and ivacaftor, but it is one FDA approved therapy that targets the issue at a cellular and molecular level as opposed to treating symptoms (29).

**CFTR Correctors**

The goal of a CFTR corrector is to restore the processing of CFTR. While CFTR potentiators work to increase the function of already existing CFTR, correctors work to increase the number of CFTR that make it to the membrane. This is a particularly popular area of research because the mutation ΔF508del, one of the most common CF mutations, is caused by an error in CFTR processing, more specifically CFTR folding. Some forms of the correctors work to down regulate cellular chaperones, whose functions are to breakdown misfolded proteins (39). Another attempted mechanism is increasing the half life of CFTR so that it has more time to travel to the cell membrane. Most of these therapies are attempting to target cellular pathways that prevent CFTR from making it to the cell membrane due its misfolded nature. Some other CFTR correctors focus on supporting the folding of CFTR. For example, trafficking assays are shown to increase the translocation of misfolded CFTR to the membrane (29).

**Gene Replacement**

Next, there is active research on gene replacement through viral and nonviral gene therapies. The focus of the gene therapy is in the lungs due to the high mortality and morbidity associated with lung pathologies that arise in CF patients. This is a difficult place to focus the studies because of the lack of success in gene transfer into the lungs caused by the high number of immunity barriers, both intracellular and extracellular (11). These studies would allow for
targeted therapies that are unique to the patient due to the complex heterozygous combinations of CFTR mutations. Similar to gene therapy, there is research examining the expression of CFTR through mRNA transduction. This would allow for the proper processing and translocation of CFTR despite mutated genes. There has been some research suggesting this may be a viable treatment, as it has been shown to restore cAMP-induced CFTR currents similar to those in healthy tissue (1).

**Translational Readthrough**

Another therapy, translational readthrough, targets premature stop codons that occur in nonsense mutations. This therapy has been explored for CF and other genetic diseases caused by nonsense mutations (29). Results have shown that therapies such as aminoglycoside antibiotics allow for the expression of full length CFTR (12). This mechanism occurs because the aminoglycoside antibiotics interact with a proof-reading portion of the ribosome and decreases its ability to properly recognize premature stop codons (29). There is also evidence that this mechanism does not cause elongation of mRNA, which was a major concern for this type of treatment (33). This treatment type has also been approved for other genetic diseases also caused by nonsense mutations, such as Duchenne muscular dystrophy and Hurler syndrome (22). There is an increase in research attempting to prevent or remedy the initial defect causing CF and many of them are showing promising potential for individualized treatment for CF and other genetic diseases.

**Respiratory Treatments**

While the basic defect treatments attempt to resolve the cause of cystic fibrosis, the rest of the treatments here focus on treating the symptoms that lead to the morbidity and mortality associated with CF. The main therapies focus on treating the lung symptoms, as those cause the
most complications for patients. These complications arise from the basic defect, in which the lack of functional chloride channel in the submucosal glands of the lungs lead to a lack of hydration in the lungs. This causes thick, difficult to move, mucus and an increase likelihood of respiratory infection and disease.

**Airway Clearance Therapy**

The first therapy is part of the daily routine of CF patients: chest physical therapy. This process commonly utilizes percussion and postural drainage. This has clinically been shown to be beneficial for most patients, however, it requires assistance and therefore adult patients often seek more independent solutions (19). This has led to chest physiotherapy vest clearance systems that work similarly to loosen mucus so that it can be better cleared by the mucocilliary escalator. This is completed by the patient wearing a vest that compresses the chest and can be used during simultaneous nebulized medication administration, allowing for a more complete treatment (44). These physical therapies, in the form most suitable for the individual patient, are a crucial daily part of every CF patients treatment. While there are few completed clinical trials about chest physiotherapy, the clinical anecdotal evidence is overwhelming and is considered standard of care (29).

Another therapy used for airway clearance is positive expiratory pressure (PEP). A PEP device is placed on the patient and he/she respires for 12-15 breaths, then the device is removed and the patient performs 2-3 forced expiratory breaths. The device provides back pressure into the patient’s lungs, causing the build-up of gas. This leads to increased clearance of mucus and functional residual volume over the 12-15 breaths. The force expiration assists in the movement of mucus. This method has shown similar results to other chest percussion therapies (20). There is still research being done on the effectiveness of each the individual airway clearance therapies, but it is evident that they are an important part of CF treatment.
**Prevention & Treatment of Respiratory Disease**

The primary cause of mortality and in CF is due to obstructive respiratory disease and therefore eradication of respiratory infection is vital. Certain types of bacteria, such as Pseudomonas, are particularly opportunistic and dangerous for CF patients (29). The treatment of these infections involve the identification of the bacteria via sputum cultures or diagnosis by symptoms and administering the proper antibiotic or specifically anti-pseudomona (44). Another issue that arises, is that CF patients often contract antibiotic resistant strains. In this case, combinations of antibiotics may be administered in order to treat the infection.

In some cases, chronic suppressive antibiotic therapies are employed in order to curb or control chronic infection. This treatment is more aggressive and can involve administering aerosolized antibiotic, such as tobramycin (TOBI). Results have shown an increase in FEV₁ in CF patients, but there is concern that this type of treatment with any aerosolized medication could lead to antimicrobial resistance. While there is no evidence of this yet, further longitudinal research is heavily anticipated (44).

**Nutrition**

The unique nutritional necessities originates from pancreatic insufficiency that occurs in 90% of CF patients (29). Nutritional requirements are highly individualized and regulated, as the variety of mutation combinations is responsible for the large variation in the pancreas’ ability to produce the proper enzymes. Similar to in the lungs, the lack of or presence of nonfunctional CFTR causes thick secretions and in the pancreas this may result in insufficient enzyme secretion. This presents as failure to thrive in infants and is remedied by daily enzyme supplements. This is part of the standard of care for CF patients (29). Similarly, Vitamin A and E are often depleted in untreated CF patients. Other deficiencies may include vitamin D, and K. All vitamin deficiencies are corrected through dietary supplementation through daily multivitamins.
Other dietary issues may arise and therefore continuous monitoring of patients nutritional status is important. This will also allow for early detection and correction of potentially dangerous malnutrition or dyslipidemias (44).
Conclusion

Cystic fibrosis is a superb example of how focused motivation can produce great results. Within the last 20 years, the advancements in the knowledge of the basic defect of CF and the possibilities for treatment have grown astronomically. This has lead to an increase in the life expectancy of CF patients of over 15 years and this is just one success that CF research has provided. The research done for CF is now applicable to multiple types of genetic diseases. The entire CF community, the researchers, physicians, patients, caretakers, consistently demonstrate perseverance that has resulted in targeted drugs that act on the specific defect in order to reverse or prevent the issues that arise from the defect.

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