Cystic Fibrosis in the Modern Era: A Personalized Medicine Perspective

Introduction

Cystic fibrosis is a debilitating, multi-system disease that stems from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (CFF, 2022-a, Section 1). This gene encodes a protein that allows for intracellular chloride to exit the cell and attract water to the extracellular space around the cell membrane (CFF, 2022-b). The pathogenic CFTR mutations prevent the protein from exchanging chloride with the extracellular environment to varying degrees and thus, the mucus in various organs becomes thicker than usual, impairing their functions (CFF, 2022-b, Section 3; The Mayo Clinic, 2022). Patients are often diagnosed with cystic fibrosis early in life thanks to expanded newborn screening practices. The initial screening checks for immunoreactive trypsinogen, then the diagnosis is confirmed by testing the salt concentration in the patient's sweat and/or conducting DNA sequencing to identify specific mutations (The Mayo Clinic, 2022). The Cystic Fibrosis Foundation (CFF) states that there are over 1,700 mutations in the CFTR gene, which have led to the 105,000 people living with cystic fibrosis worldwide (CFF, 2022-a). Discovery of these mutations is a triumph of modern medicine and has led to greatly improved life expectancy in recent years, with an estimated 1-2% annual mortality based on European records (ERS, 2022).

Around 1954 the life expectancy of someone with cystic fibrosis was only 4-5 years, but as diagnostic methods and treatments have improved, the life expectancies have too (McBennett et. al., 2021). In 2019 the predicted life expectancy of someone born with cystic fibrosis was a remarkable 48.4 years (McBennett et al., 2021). However, the increase in life expectancy creates a few dilemmas for patients. As patients live longer the severity of the disease and its associated complications increase, leading to greater costs of care (Grosse, 2018). Grosse et al. performed an analysis of US healthcare expenditure for patients from 2010-2016 in which they found patient spending increased from $67,000 to $131,000 (2018). In this study, they also found that spending increased greatly from 2014-2016 due to the development of specialty medications, like ivacaftor, which target specific CFTR mutations.

There are currently 5 classifications of CFTR mutations which are based upon the effect of the mutation, and these classifications require specific treatments (CFF, 2022-j). Ivacaftor is common among the groups because it causes the 'gate' of the CFTR protein to allow for more chloride to pass through, effectively compensating for non-functional CFTR or insufficient CFTR levels (CFF, 2022-j). This drug is also the main line of therapy for those with "gating mutations". The most common mutation, F508del, is part of the protein processing class. Protein processing mutations result from incorrect or missing amino acids in the CFTR protein which prevent it from maintaining the proper functional shape. The treatment for this class is a combination of elexacaftor, tezacaftor, and ivacaftor that stabilize the protein in its correct shape

and allow it to function (CFF, 2022-j). The last class with a treatment is one in which there are less CFTR proteins on the cell surface than necessary. This class has two treatments: Ivacaftor (Kalydeco) and Symdeko (CFF, 2022-j). The last two classes are protein production mutations and conduction mutations. These currently have no FDA approved therapies, but there is active research on the topics (Rafeeq & Murad, 2017; Chaudary, 2018). General treatment for cystic fibrosis consists of bronchodilators to promote mucus clearance, pancreatic enzyme supplements to aid in digestion, and many antibiotics to treat frequent lung infections (CFF, 2022-a).

Each patient experiences the disease in a unique way because symptoms may be influenced by both genetic and non-genetic factors. The formal classification of the disease is as a monogenic recessive disorder, but recent studies have revealed that there are other genes – called modifiers – which cause variation in phenotypes (Knowles & Drumm, 2012). Apart from genetics, there are a few factors under examination: environment, epigenetics, and the microbiome. One study by Chen et al. showed that patients with cystic fibrosis exhibit a unique epigenetic profile in which there is general hypo-methylation in macrophages (2018). They go on to conclude that this is one way in which the immune system is dysregulated in cystic fibrosis. Environmental factors are broad and can range from air quality to socioeconomic factors. In a study by Ong et al., socioeconomic status and smoke exposure were examined for their effects on pediatric patient outcomes (2017). The conclusion of the study was that smoke exposure and lower socioeconomic status were both associated with worse patient outcomes. The microbiome of both the lungs and small intestine of cystic fibrosis patients are under investigation to determine the relationship between dysbiosis and phenotype (Huang & LiPuma, 2016; Rogers et al., 2016). While the relationship is still under investigation, the studies did show that the microbiomes are different in cystic fibrosis patients compared to the normal flora. This glimpse into the factors affecting the disease reveal that cystic fibrosis is a multi-faceted disease wrought with opportunity to advance and improve quality of life and outcomes for patients.

Cystic Fibrosis and Family Planning

Preconception Carrier Testing

One's decision to have children naturally, by in vitro fertilization, by a donor, or by adopting may be impacted by the results of carrier testing. One may be worried about the quality of life for their child, and although the average lifespan of someone with cystic fibrosis is about 37, studies show that patients' quality of life varies widely (ACOG, 2020-a; Abbott et al., 2015; Dill et al., 2013; Pittman et al., 2011; Linnemann et al., 2022). Testing gives prospective parents information which empowers them to make an informed decision during family planning (ACOG, 2017).

Cystic fibrosis is one of the diseases that the American College of Obstetrics and Gynecologists recommends for carrier testing, preferentially before one is pregnant (ACOG, 2017). Screening is completed by collection of a blood sample, then capturing DNA from the blood to test for the common variants which cause cystic fibrosis (Sonora Quest Laboratories, 2022; UCSF, 2022-b). Testing may return a false negative in the event that a patient has a rare mutation in the CFTR gene, and patients should be notified of this in advance of testing (Sonora Quest Laboratories, 2022; UCSF, 2022-b). It is also important to note that the prevalence of

mutations commonly tested are most often present in the non-Hispanic European population and coverage of testing varies by ethnicity (ACOG, 2020-a; Sonora Quest Laboratories, 2022; UCSF, 2022-b).

Prenatal Testing

Prenatal testing for cystic fibrosis is appropriate, but like all genetic tests, is optional to parents. With the information gathered from prenatal testing parents may choose to terminate the pregnancy for the aforementioned quality of life anxieties or to avoid the psychosocial caregiver burden associated with cystic fibrosis (Daly et al., 2022). A positive prenatal test gives parents time to collaborate with physicians and prepare themselves to care for a child with cystic fibrosis.

Prenatal Testing Methods

Chorionic villus sampling and amniocentesis can be used for early diagnosis of cystic fibrosis in a fetus (ACOG, 2020-a; UCSF, 2022-b). Chorionic villus sampling is completed from 10-13 weeks of pregnancy and involves removal of a small sample of the placenta (UCSF, 2022-c). Amniocentesis is completed later on, at 15-20 weeks, and is performed by aspirating a small amount of amniotic fluid with a needle (UCSF, 2022-a). Pre-implantation genetic testing is also viable for those using in vitro fertilization for conception (ACOG, 2020-b). Ultrasound results can indicate further testing for cystic fibrosis is required, but they are not used for diagnosis (Scotet et al., 2002); these results have been shown to be clinically significant for early identification of cystic fibrosis in patients of non-European descent (Mekki et al., 2021). Noninvasive prenatal testing is another prenatal testing method, typically used for aneuploidy detection, that is under development for use in detecting of cystic fibrosis (Hill et al., 2015; Jeppesen et al., 2021).

Pregnancy with Cystic Fibrosis

Women with cystic fibrosis are able to bear healthy children and are at least risk when they are at their healthiest (CFF, 2022-g; Geake et al., 2014). In order to reach this level of health and maintain it throughout the pregnancy, one must collaborate with their medical team to manage their lung function, nutrition, blood glucose, and liver health (CFF, 2022-g; Geake et al., 2014). These are all aspects affected by cystic fibrosis that can be exacerbated during pregnancy, so it is critical that these aspects be closely monitored. Blood glucose should be monitored because pancreatic function is drastically reduced in some patients, leading to either cystic fibrosis related diabetes or higher risk of developing gestational diabetes (CFF, 2022-e; CFF, 2022-g; Geake et al., 2014). Physicians may cease CFTR modulator therapy during pregnancy as a precaution because no studies have included pregnant patients in their cohorts to date (Taylor-Cousar, 2020; Geake et al., 2014). There are some recorded pregnancies where the mother was taking modulators throughout the pregnancy and no adverse events occurred, but this is a small sample and does not involve the rigor required to determine safety of modulators during pregnancy (Taylor-Cousar, 2020). The most common adverse events in pregnancies where the mother has cystic fibrosis are pre-term delivery and low birth weight, but most children have APGAR scores within normal limits and show no signs of deficit (Geake et al., 2014).

Genomic Testing for CFTR Variants in Healthy People

To pursue genomic testing for cystic fibrosis one may consider consulting a physician for a genetic panel so they may provide guidance, but there are also direct-to-consumer tests available, like the panel from 23andMe listed in Table 1.

Table 1: A compilation of some genomic testing panels available for the CFTR gene, what is tested for, and the companies which supply them. The 23 core mutations refer to the 23 most common CFTR mutations recommended for testing by the American College of Medical Genetics.

Test Name	Gene	Test Focus	Mutations Tested For	Company	Product Website
Cystic Fibrosis Common Mutation Panel	CFTR	Mutations	23 core 37 others	Institute for Genomic Medicine (IGM) Clinical Laboratory	https://www.nationwidechildrens.org/specialties/laboratory-services
Cystic Fibrosis Mutation Panel	CFTR	Mutations	23 core 83 others	Mayo Clinic Laboratories	https://www.mayocliniclabs.com/order-tests/index.html
Cystic Fibrosis Diagnostic Mutation	CFTR	Mutations	23 core 37 others	Michigan Medical Genetics Laboratories	https://www.pathology.med.umich.edu/handbook/#/details/972
CFTR Deletion/ Duplication Analysis	CFTR	Deletion/ Duplication Analysis	N/A	Michigan Medical Genetics Laboratories	http://www.pathology.med.umich.edu/handbook/
CFTR Gene Sequencing	CFTR	Exon sequencing	N/A	Michigan Medical Genetics Laboratories	https://www.pathology.med.umich.edu/handbook/#/details/1100
CFTR Targeted Mutation Analysis for Cystic Fibrosis	CFTR	Mutations	23 core 9 others	Molecular Diagnostics Laboratory	http://www.dukemolecular.org/

Test Name	Gene	Test Focus	Mutations Tested For	Company	Product Website
CFvantage Cystic Fibrosis Expanded Screen	CFTR	Mutations	23 core	Quest Diagnostics Nichols Institute San Juan Capistrano	https://testdirectory.questdiagnostics.com/test/home
23andMe Health + Ancestry Service	CFTR	Mutations	23 core 6 others	23andMe	https://www.23andme.com/topics/carrier/cystic-fibrosis/

Lifestyle Changes for Carriers and the Newly Diagnosed

The push for widespread newborn screening has made early detection of cystic fibrosis much more common, but there are adults living with the condition that have yet to be diagnosed (NHLBI, 2022). If an adult were to learn they have cystic fibrosis, they would need to avoid or cease smoking, develop regular handwashing habits, create a fitness regiment, and it would be helpful to create a schedule for medication administration (CFF, 2022-A). Dietary modifications, such as focusing on nutrient and calorie dense foods are appropriate as well due to CF's impact on digestion and nutrient absorption (CFF, 2022-A). Those with CF also undergo increased testing as complications of the disease arise. While they may not need to do any more genetic tests, they may undergo ultrasounds, x-rays, CT scans, glucose monitoring, pancreatic tests, lung function tests, and sputum cultures (CFF, 2022-A). The initial testing panels will check the CFTR alleles a patient has, and if they have common variants then these will suffice (Johns Hopkins Cystic Fibrosis Center, 2022). If the initial testing does not return diagnostic evidence, then more in-depth testing like exome sequencing or targeted CFTR gene sequencing may be performed (Johns Hopkins Cystic Fibrosis Center, 2022). No lifestyle modifications would be necessary for a carrier, but family planning decisions may be influenced.

Newborn Screening Process for Cystic Fibrosis

Newborn screening is performed nationwide for CF, but the exact method varies by state (NHLBI, 2022; Johns Hopkins Cystic Fibrosis Center, 2022). The first step in all cases is a blood spot test to check for high levels of immunoreactive trypsinogen (IRT) in the blood (NHLBI, 2022). In Maryland, the second step would be a second IRT test, followed by a sweat test in the event of another positive IRT test (Johns Hopkins Cystic Fibrosis Center, 2022).

Testing After a Relative is Diagnosed

One may wish to undergo carrier screening if a sibling or parent test positive for cystic fibrosis or if one plans on having children. As previously mentioned, carrier screening allows prospective parents to assess the risk of passing on pathological mutations to their children. The typical testing panel looks for the 23 variants recommended by the ACMG, so if they have a rare variant then it would not be reported (NHLBI, 2022). There are other panels, examples of which

are in Table 1, that include an expanded list of screened mutations or options to sequence the whole gene in cases where the family member's mutation is not included on panels.

Cystic Fibrosis and the Environment

Environmental factors affecting cystic fibrosis outcomes include food accessibility, mental health, air pollution, and exercise habits. Pollution, nutrition, and exercise habits all directly influence general wellbeing or lung health in particular, while mental health indirectly affects outcomes through the management of care.

Studies have found correlation between air quality and frequency of pulmonary exacerbations, forced expiratory volume (FEV), and lung infection frequency in cystic fibrosis patients (Farhat & Barrios, 2022; Jassal et al., 2013; Psoter et al., 2015). Air quality may be influenced by a variety of factors, but the main culprits are microscopic particulate matter, ozone, carbon monoxide, and secondhand smoke (Farhat & Barrios, 2022). The pollutants interact with the inflammasome within the patients' lungs, which increases inflammation and reduces pulmonary function, as measured by FEV (Farhat & Barrios, 2022; Jassal et al., 2013). In particular, apoptotic pathways show increased expression upon exposure to particulate matter by the activation of caspase-9 and PARP-1, ozone exposure reduces CFTR expression and function via the STAT1 path and causes bronchial epithelial apoptosis by downregulating SERCA2 production, and secondhand smoke reduces CFTR activity due to the direct effect of chemicals like acrolein on the CFTR protein (Kamdar et al., 2008; Qu et al., 2009; Rasmussen et al., 2020) There are some genetic variants related to inflammatory factors like interleukin 1B, 8, and 10 which increase severity of exacerbations in patients with the variants, but they are not required for an increase in exacerbation frequency or magnitude due to pollutant exposure (Butnariu et al., 2021). These pollutants gain the effect of increased infection frequency due to their effect on airway clearance (Farhat et al., 2022; Rasmussen et al., 2020).

Nutrition and exercise management are extremely important for patients with cystic fibrosis, as digestion and absorption of nutrients are impaired by cystic fibrosis, and careful exercise plans are associated with longer lifespans in cystic fibrosis patients (Jassal et al., 2013; Shei et al., 2019). A study by Jassal et al. indicated that living in or close to a 'food desert' – characterized by low income and low access to healthy foods – increases the likelihood of a patient having an abnormal BMI, whether that be underweight or overweight, and poor lung function compared to other cystic fibrosis patients (2013). Shei et al. evaluated the concept of exercise intolerance due to progression of cystic fibrosis and its treatment (2019). Despite the positive effects of exercise on patients' prognosis, it is foundationally a habit and takes effort to establish a routine for it. Treatment of cystic fibrosis may involve hospitalizations where there is little emphasis on exercise and the maintenance of proper nutrition is imperative for patients to have the energy to exercise, but both of these factors are inherent to their lifestyle and make it difficult to maintain the habit of exercising. This management of care can be further confounded by poor mental health outcomes in the cystic fibrosis population. Platten et al. found that their patient sample had higher incidence of clinically significant mental health symptoms than the general population and that an increase in mental health symptoms was correlated with lower quality of life (Platten et al., 2013). The interconnected nature of mental health, exercise, and nutrition makes it so that a deficit in one leads to a decline in the others, and a decline in any of them leads to worse outcomes for cystic fibrosis patients.

The Impact of the Omes

Advances in biology, chemistry, engineering, and computer science have allowed for sophisticated analysis of specific systems as they relate to cystic fibrosis and other diseases. Collectively, these systems are referred to as the Omes due to their shared suffix, -ome. In this paper, studies of the epigenome, transcriptome, proteome, metabolome, and microbiome will be discussed as they relate to cystic fibrosis diagnosis, treatment, or outcomes.

Epigenome

The field of epigenomics has led to a few discoveries of disrupted methylation within the cystic fibrosis population and there are many more discoveries in waiting. The epigenetic changes which will be discussed are promoter methylation in macrophages, post-transcriptional methylation control of the CFTR protein in bronchial epithelial cells, and downregulation of epithelial sodium channel (ENaC) by promoter methylation modulation in bronchial epithelial cells.

The process of autophagy is carried out by all cells and one of its purposes is to reduce microbial burden within cells (Caution et al., 2019). In a study by Caution et al., they found that hypermethylation of a promoter for the gene Atg12 led to decreased protein production in cystic fibrosis patients (2019).The Atg12 gene encodes proteins essential to autophagy, so this reduction in expression leads to reduction in autophagy, increasing susceptibility to infection. They tested a drug by the name epigallocatechin-3-gallate in mouse models and found that it reduced methylation at the Atg12 promoter and rescued its production. There is still active research surrounding this drug and alternatives.

The post-transcriptional study was carried out by D'Amore et al. in order to prevent F508del CFTR proteins from beginning the degradation cycle, thus increasing the effectiveness of modulatory drugs (D'Amore et al., 2022). The group identified lysine residues on the CFTR protein that could be ubiquinated, leading to a degradation cascade, but when these lysines were methylated, the proteins were not destroyed. To preserve methylation at these sites they used siRNAs against two demethylases, KDM2A and KDM2B. Their results show that prevention of ubiquination through downregulation of KDM2A and KDM2B improves functional recovery of F508del CFTR proteins through the typical modulatory therapy.

ENaC is a protein that acts in conjunction with CFTR in the healthy individual to maintain adequate hydration at the cell surface (Blaconà et al., 2022). When CFTR is dysfunctional then the activity of ENaC is underregulated and the cell surface becomes dehydrated due to ENaC activity. ENaC has three subunits, their production was the target of Blaconà et al (2022). The group found that the SCNN1A and SCNN1B genes had CpG islands around their promoters, indicating that they could be epigenetically controlled through methylation around the promoter. They used a combination of curcumin, a chromatin condenser, and S-adenosyl methionine, known to induce methylation, to downregulate expression at the aforementioned genes. Their results confirmed that ENaC can be suppressed via chromatin modulation and DNA methylation and that it is a viable target for therapies. Suppression of

ENaC production led to a partial restoration of cell-surface hydration. They did not test for off-target effects of this treatment, so more studies are needed before it can be used therapeutically.

Transcriptome

The advent of transcriptomics has led to much greater characterization of phenotypic changes in cystic fibrosis patients. Those with the disease experience transcriptional dysregulation in various cells, and these irregularities are the subject of therapeutic research. One of these pathological changes is seen in neutrophils. Typically, neutrophils undergo transcriptional changes in response to whatever tissue they are entering to mount an immune response (Margaroli et al., 2021). In the case of cystic fibrosis, Margaroli et al. discovered that exposure to the CF microenvironment causes neutrophils to downregulate immunomodulatory genes and upregulate metabolic genes, leading to low antimicrobial activity in the lungs. They treated these neutrophils with the RNA polymerase inhibitor alpha-amanitin and had success in restoring bactericidal activity in vitro. In general, the lung epithelial cells have been shown to have altered transcriptional activity compared to the same cells in healthy patients (Clarke et al., 2015; Carraro et al., 2021). Clarke et al., compared transcriptomes between cystic fibrosis and other respiratory disorders such as COPD and found overlap among affected pathways (2015). They were also able to identify some CFTR regulators, and by using siRNA, determined their effect on CFTR transcription and function. Carraro et al. compared transcriptomes of healthy bronchial epithelial cells against bronchial epithelial cells with pathogenic CFTR mutations (2021). They found that basal epithelial cells, those responsible for dividing and producing other epithelial cells, were at a decreased level in the diseased cell populations and the secretory cells were acting in a stress-related manner compared to healthy tissue, resulting in more secretions and inflammation (Carraro et al., 2021).

Proteome

Cystic fibrosis as a whole can be attributed to malfunction of the CFTR protein, and recent research has been focused on understanding how this protein is altered post-translationally to elucidate treatment opportunities (Liessi et al., 2020). Protein interactions have mostly focused on the most common variant, F508del-CFTR, to identify what proteins are involved with its processing in wild type and pathogenic cells (Wang et al., 2006; Pankow et al., 2015). These fundamental analyses unveiled separate classes of proteins involved in folding, RNA processing, and degradation of the CFTR protein (Liessi et al., 2020; Pankow et al., 2015). The field is still undergoing rapid advancement and studies are becoming more focused on the interactions between CFTR and the endoplasmic reticulum or cell membrane rather than looking at the whole interactome within a cell (Liessi et al., 2020). These various protein interactions and studies focused on functional changes in CFTR have led to the modulator therapies recently approved for clinical use, and the application of proteomics is far from saturated.

Metabolome

The metabolome of cystic fibrosis is under active investigation, but the validity of various biomarkers have yet to be determined (Chandler & Esther, 2022). The most widely agreed upon metabolic markers are immunoreactive trypsinogen and chloride levels in sweat, which are implemented in newborn screening for cystic fibrosis throughout the U.S. (Macedo et al., 2017). Due to the existing collection of these samples, Macedo et al. analyzed the metabolome of sweat samples to further evaluate potential biomarkers. They discovered that glutamine and asparagine were positively associated with chloride levels and positive cystic fibrosis tests, due to their reliance upon chloride transport systems for their own transport (Macedo et al., 2017). Sputum and bronchial lavage fluid have been the main methods of sample collection for other metabolomic assays, but due to the recent success of modulator therapies these samples may not be so readily available (Chandler & Esther, 2022). Chandler and Esther estimate that the future of metabolomic assays is through less invasive means, like breath analysis, and it is likely that sweat samples will gain favor as well (Chandler & Esther, 2022).

Microbiome

Although cystic fibrosis arises from a genetic mutation in the CFTR gene, the composition of a patient's intestinal and lung microbiomes is indicative of the severity of their disease and contributes to personal variations in the disease. One of the most common pathogens, found in over 60% of patients with cystic fibrosis is *Pseudomonas aeruginosa* (CFF, 2022-h). This pathogen is opportunistic, and essentially omnipresent, so due to patients' inability to effectively clear their airways, the microbe takes hold in the lungs. Though, this microbe is not alone. In fact, one study found that patients have about 86 distinct microbial taxa linked to the disease, and they found 598 distinct taxa in their sample of 297 patients (Cuthbertson et al., 2020). The composition of one's lung and gut microbiome varies due to environmental factors regularly, but a main contributor to its diversity in cystic fibrosis patients is the continued use of antibiotics while battling infections (Cuthbertson et al., 2020; Françoise & Héry-Arnaud, 2020). Both studies have shown that microbial diversity decreases as disease severity increases and the typical point at which diversity starts to decline is around age 11 (Cuthbertson et al., 2020; Françoise & Héry-Arnaud, 2020). The microbiota shift from the normal anaerobic species normally found in the lungs to aerobic bacteria as severity increases as well. The gut and lung microbiomes 'talk' as well. Bacteria in the gut are responsible for producing short-chain fatty acids which are critical to immune function (Françoise & Héry-Arnaud, 2020). The decrease in these microbes causes further lung inflammation and predisposition to lung infection. Overall, the lung and intestinal microbiomes are rife with opportunity to improve outcomes through analysis. Antibiotic prescription can be considered alongside preferred microbiota colonization to prevent decreasing diversity and preserve lung function. Patient outcomes may also be estimated based on bacterial cultures – the less diverse cultures indicate worse outcomes.

Immune System Modulation in Cystic Fibrosis

The immune system response is widely dysfunctional in cystic fibrosis due to modifications in the activity of macrophages, neutrophils, and T cells (Khoury et al., 2018). Increased inflammation in the lungs is a major factor in disease progression– damaging healthy tissue, impairing macrophage response, and prolonging infection (CFF, 2022-i; Hofer et al., 2014). Unfortunately, the inflammation is due to an immune response gone haywire.

The CFTR protein regulates ion concentrations within the cell and an organelle called the phagosome, which is present in neutrophils and macrophages (Zhou et al., 2013; Hofer et al., 2014). A lack of a functional phagosome leads to the inability to present a pathogen's antigen on the MHC II receptors, which stunts the progression of an immune response from innate to adaptive. It has also been shown that increased inflammation results in downregulation of HLA-DQ – an MHC II protein – in macrophages because of lower transcriptional activity (Hofer et al., 2014). They established that this is at least partially due to reduced activity of the CIITA transcription factor, which normally induces HLA-DQ transcription in the presence of IFN-gamma. Autophagy is further inhibited by hypermethylation of the Atg12 gene promoter, which represses expression of a protein component involved in a complex with other ATG proteins (Caution et al., 2019). Pathogen recognition is also impaired for macrophages because there is reduced expression of membrane-bound CD11b and TLR-5 (Simonin-Le et al., 2013). On another front, macrophages are adding to the inflammation by releasing cytokines and sCD14, trapping themselves in a positive feedback loop (Simonin-Le et al., 2013).

Neutrophils are subject to the same detrimental phagocytic and pathogen recognition effects as macrophages because they rely upon CFTR in the same way (Zhou et al., 2013; Hofer et al., 2014). Neutrophils have an additional issue relating to their degranulation – the method by which they disseminate functional proteins extracellularly. Degranulation is reduced for neutrophils with the F508 or G551D variants of CFTR, but only for select proteins like MMP-9 and lactoferrin, the latter of which is important for preventing biofilm formation (Pohl et al., 2014). Exocytosis is impaired by the ion imbalance created by dysfunctional CFTR, specifically by destabilizing the complex formed between Rab27a and GTP because of a magnesium ion scarcity (Pohl et al., 2014). Interestingly, degranulation is not impaired in all cases and neutrophils exhibit increased exocytosis of a protease called neutrophil elastase which wreaks havoc on proteins in the cystic fibrosis lung environment (Khoury et al., 2018). Prolonged exposure and release of neutrophil elastase is associated with decreasing lung function, partially due to alveolar injury through elastin digestion (Khoury et al., 2018; Voynow et al., 2021). Elastase causes further inflammation and airway occlusion by upregulating various mucins, causing macrophages to release TNF-alpha and IL1-beta, and recruiting more neutrophils to the area (Voynow et al., 2021).

T-cells further contribute to the inflammation in cystic fibrosis due to a bias towards Th2, Th17, and Th22 cells in the immune response and a decrease in regulatory T-cells known as Tregs (Hector et al., 2015; Khoury et al., 2018; Zhang & Zhang, 2020). It has been shown that infection by *P. aeruginosa* exacerbates the inflammatory properties of T-cells and is responsible for the decrease in Tregs, but the exact mechanism is still unknown (Hector et al., 2015). Th2

cells release increased amounts of IL-4 and IL-13 cytokines, Th17 cells release IL-17 cytokines, and Th22 cells produce IL-22 which is typically associated with repair of fibrosis, but its role in cystic fibrosis is unclear (Zhang & Zhang, 2020).

Immunological Potential for Improvement

The vast array of immune malfunctions provides multiple avenues for improvement in the management and evaluation of a patient's disease progression. Ivacaftor is a recently approved drug for the treatment of various CFTR mutations, and it works by improving the ability of the CFTR protein to transport chlorine ions (CFF, 2022-d). This has the benefit of improving mucus mobility in patients, but it also improves the function of phagocytic cells like macrophages and neutrophils by allowing the phagocytes to function properly (Zhou et al., 2013; Hofer et al., 2014). Another example of a promising drug is epigallocatechin gallate, commonly known as EGCG, which has been shown to improve CFTR protein function and cell-surface expression through epigenetic mechanisms in macrophages, sweat ducts, and nasal epithelial cells (Tosco et al., 2016; Caution et al., 2019). Furthermore, the release of each of the aforementioned cytokines and proteins is specific to cell type, so evaluation of the concentration of these molecules can potentially provide information about the disease progression and impending exacerbations. The diagnosis of cystic fibrosis remains unaffected at this point due to new immunological information, leaving the sweat test, immunoreactive trypsinogen, and genetic testing as the gold standard for diagnosis (Mayo Clinic, 2022-a).

Recent Clinical Trials

There are a variety of clinical trials underway for cystic fibrosis, and most of them focus on restoring CFTR function. This is immunomodulatory by restoration of phagocytic activity and pathogen recognition as mentioned previously.

One of the common CFTR modulators under investigation is ivacaftor. This is currently used clinically for a variety of CFTR mutations but is particularly effective for gating mutations like G551D (De Boeck et al., 2014). In one study, the long-term effects of ivacaftor therapy in children from 0-2 years old are under investigation (Vertex Pharmaceuticals Incorporated, NCT03277196). There are 86 patients enrolled in the study and they all have mutations indicated for ivacaftor therapy. Patients are either part of the treatment group, where they received ivacaftor every 12 hours for 96 weeks, or part of the observational group which receives no treatment. Safety is evaluated by frequency of adverse events and serious adverse events from the period of the baseline reading up to 24 weeks after the last ivacaftor treatment a patient received.

Another study in CFTR modulation was conducted by the Hadassah Medical Organization in Israel, focusing on the effects of EGCG and tocotrienol in patients with splicing mutations in the CFTR gene (Hadassah Medical Organization, NCT00889434). This trial consisted of 7 participants that each underwent a series of 3 treatment regiments to evaluate which was best. First, patients were treated with EGCG for 28 days then given 14 days for the drug to exit their system. This was repeated once more before moving into the next treatment. Secondly, a 28-day

treatment cycle was conducted with tocotrienol followed by another 14-day period without treatment. Lastly, a 28-day treatment period with both drugs was conducted. The effect of therapy was measured by chloride secretion via transepithelial potential difference and evaluation of lung function through forced expiratory volume, forced vital capacity, and maximal expiratory flow. There is no publication for this study, and it has not been updated within the past 5 years, unfortunately.

Pharmacogenomics

Cystic fibrosis may be a recessive monogenic disease, but its management relies upon many medications that may be impacted by the genome. Table 2 summarizes the types of drugs which are used in the management of cystic fibrosis and how genomic variants may influence their function. The variant information contained within this section was collected using the PharmGKB website (Whirl-Carillo et al., 2021).

Table 2: Types of medications used to treat cystic fibrosis with representative drugs from each category. The number of genomic variants and their associated impact on the bolded drug's function are included, if available. The "Variant Number" column may contain the acronym NKV, which stands for "no known variants".

Drug Classification	Example Drugs	Variant Number	Variant Effect	Sources
Targeted CFTR Modulators	**Ivacaftor** Elexacaftor Tezacaftor Lumacaftor	33	Only those variants are treatable with this drug	Mayo Clinic, 2021; Thursfield and Davies, 2013
Antibiotics	**Tobramycin** Colistin Ciprofloxacin Levofloxacin Azithromycin Rifampin	2	Increased risk of hearing loss	Tacceti et al., 2021; Chmiel et al., 2013; CFF, 2022-f
Anti-Inflammatory Agents	**NSAIDs (Ibuprofen)** Prednisone	3	Decreased drug metabolism	CFF, 2022-f
Mucolytics	Dornase alpha Mannitol Hypertonic saline	NKV	N/A	Henke & Ratjen, 2007; CFF, 2022-i
Bronchodilators	**Albuterol** Levalbuterol hydrochloride	1	Varied albuterol binding affinity	CFF, 2022-c

Drug Classification	Example Drugs	Variant Number	Variant Effect	Sources
Pancreatic Enzymes	Creon Zenpep Pancreaze Ultresa Violate Pertzye	NKV	N/A	FDA, 2012
Stool Softeners	**Polyethylene glycol** Lactulose Sodium docusate Senna glycoside	1	Risk of hemolysis	CFF, 2022-k; Green et al., 2017
Stomach Acid Reducers	**Omeprazole** Ranitidine Esomeprazole Nizatidine	3	Varied drug metabolism and efficacy	CFF, 2022-k

Concluding Thoughts

For a monogenic disease, cystic fibrosis exhibits surprising complexity due to the downstream effects of CFTR protein dysfunction. With wide-reaching effects spanning from reduced cell surface hydration to impaired immune system function resulting from a chronically inflamed lung environment, the potential for improvement in its management may take many forms. It is my opinion that an advanced understanding of the proteome and microbiome will have the greatest impact on quality of life for cystic fibrosis patients in the near future. As previously discussed, lung microbiome composition is a strong indicator of disease progression, so selective targeting and modification of the lung flora will attenuate severity if implemented correctly. Proteomics has the potential to characterize the misfolded CFTR proteins and develop chaperone molecules to either correct their folding or aid in transporting them to their final destination. CFTR mutations result in protein instability, so proteomics is truly the crux of the matter. Great progress has been made so far in the form of ivacaftor, elexacaftor, tezacaftor, and Symdeko, but there is still much suffering to relieve and ample opportunity to do so.

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