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Review article

Systematic Review of Emerging Technologies in Cystic Fibrosis Treatment: Gene Therapy and CRISPR Strategies for the Future

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Abstract: The current systematic review aimed to evaluate research on cystic fibrosis (CF), a genetic disease that affects multiple organs, particularly the lungs, and is associated with high morbidity and mortality. A total of 7831 relevant studies were identified from search databases, and 27 studies were ultimately considered appropriate for review after applying eligibility criteria including three longitudinal studies and the remaining cross-sectional ones. All studies included a healthy control group, with a combined total of 1839 individuals with CF and 2178 controls. The age range varied across studies, but the majority were carried out in adults. The studies had different aims, including evaluating and comparing different techniques for gene therapy and CRISPR, and assessing changes in body nutrition status. Other studies focused on the evaluation of lung function, inflammation, and clinical parameters. Animal models have played a crucial role in advancing CF gene therapy. Various animal models have been developed, including pigs, ferrets, rats, zebrafish, and sheep, each with its advantages and limitations. The CF pig model has facilitated the measurement of CFTR correction in vivo, which enhances the correlation of CFTR expression and transportation of Cl- and HCO3 as a result decreases the implication of cystic fibrosis in gene therapy. Gene editing technologies, such as CRISPR/Cas9, have emerged as promising approaches to modifying nucleic acid sequences in CF research. These tools hold the potential to repair the endogenous CFTR gene and restore its function, but efficient in vivo gene delivery remains a significant challenge. Assessing changes in body composition can provide valuable information on the effects of gene therapy or CRISPR on the overall health of CF patients. The proper evaluation of nutritional composition in the CF treatment is essential, as current therapies such as CFTR modulators primarily target the respiratory system and may not fully address the systemic effects of the disease. The CRISPR techniques and gene therapy treatment have the potential to provide more comprehensive and long-lasting treatments for CF and the assessment of body composition changes is essential for future clinical trials.

Keywords: Cystic Fibrosis, genetic disease, systematic review, morbidity, mortality, gene therapy, CRISPR/Cas9, CFTR gene, CFTR modulators, systemic effects, clinical trials, nucleic acid modification, endogenous CFTR repair, health assessment, respiratory system.

1. Introduction

Cystic fibrosis (CF) is a genetically inherited disease found primarily in the lungs (Alton et al., 2013). In healthy individuals, the CFTR protein helps maintain a balance of salt and water in the body's cells and tissues. However, in individuals with cystic fibrosis,

a genetic disorder caused by mutations in the CFTR gene, the CFTR protein can't perform its function correctly, as a result of the accumulation of thick mucous in the lungs and other vital organs (Alton et al., 2013). The other side effect of a Mutation in the CFTR gene can cause serious health issues, such as the congenital bilateral absence of the vas deferens (CBAVD), which affects male fertility, and chronic bronchitis and bronchiectasis, which are respiratory conditions that affect airways (Stuhrmann & Dork, 2000). Cystic fibrosis (CF) is one of the most common inherited disorders in the United States. According to the Cystic Fibrosis Foundation, approximately 30,000 people in the United States have cystic fibrosis. The prevalence of cystic fibrosis in the United States is estimated to be about 1 in 3,500 live births (Ruzal-Shapiro, 1998). However, the prevalence of the disease can vary depending on different factors, such as ethnicity and geographic location. the disease is more common in individuals of European descent, with a prevalence of 1 in 2,500 live births, compared to individuals of African American or Asian descent, with a prevalence of 1 in 17,000 and 1 in 31,000 live births, respectively. Geographic location can also play a role, with higher rates of cystic fibrosis reported in certain regions of the country, such as the Midwest and Northeast (Dongarwar et al., 2022). On another hand, the prevalence of cystic fibrosis (CF) in Europe varies by country and region. According to the European Cystic Fibrosis Society Patient Registry, the average prevalence of CF in Europe is approximately 1 in 10,000 live births, with some variation depending on the country and region. For example, in Northern and Western Europe, the prevalence of CF is higher, with rates ranging from 1 in 5,000 to 1 in 9,000 live births. In Southern and Eastern Europe, the prevalence is lower, with rates ranging from 1 in 15,000 to 1 in 25,000 live births (Mehta et al., 2010). Additionally, the frequency of specific CF mutations can vary by region and ethnic group. For example, the most common CF mutation in Northern and Western Europe is the F508del mutation, while in Southern Europe, the R1162X mutation is more common (Bobadilla et al., 2002). Gene therapy is a promising treatment approach for cystic fibrosis (CF) that aims to correct the underlying genetic defect responsible for the disease (Quintana-Gallego et al., 2014). CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which provides instructions for producing the CFTR protein. Gene therapy for CF involves introducing a healthy copy of the CFTR gene into cells to replace the mutated gene. There are several approaches to gene therapy for CF, including viral and non-viral vectors. Viral vectors are modified viruses that are used to deliver healthy CFTR genes to cells in the lungs (Derichs, 2013). The most used viral vectors for CF gene therapy are adeno-associated viruses (AAVs) and lentiviruses. These viruses are modified so that they cannot cause disease, but they can still enter cells and deliver healthy genes. Non-viral vectors use other methods to introduce healthy CFTR genes into cells, such as lipid nanoparticles or plasmids (Li & Samulski, 2020). These methods are less efficient than viral vectors, but they may be safer and more easily scalable. Once the healthy CFTR gene is introduced into cells, it can produce the normal CFTR protein, which can regulate salt and fluid movement in and out of cells. This can help to reduce the build-up of thick, sticky mucus in the lungs and other organs (Derichs, 2013). Gene therapy for CF is still in the early stages of development, and several challenges need to be overcome before it can become a standard treatment. These challenges include the development of safe and efficient gene delivery methods, the need for long-term expression of the healthy gene, and the potential for immune reactions to gene therapy. Despite these challenges, gene therapy for CF shows great promise and ongoing research and clinical trials are exploring new approaches to this treatment. If successful, gene therapy could provide a cure for CF by addressing the underlying genetic defect responsible for the disease (Fajac & Wainwright, 2017)

In the context of CF, CRISPR could be used to edit the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which provides instructions for producing the CFTR

protein. CF is caused by mutations in the CFTR gene, which result in a dysfunctional CFTR protein that is unable to regulate salt and fluid movement in and out of cells as a result creates mucous in the lungs and other organs as well (Schneider-Futschik, 2019). CRISPR could be used to correct the genetic mutations in the CFTR gene, which would allow cells to produce a normal, functional CFTR protein. This could potentially eliminate the symptoms of CF and provide a cure for the disease (Chen et al., 2021). The current research aims to examine the status of cystic fibrosis, including potential treatments utilizing gene therapy and CRISPR, as well as their impact on the survival of individuals with compromised lung function resulting from recurrent infections.

2. Methodology

Search Criteria

The systematic review's protocol in the supplementary material was generated hereby following the guidelines of reported items and the review's outcomes were then presented following the PRISMA checklist (Moher et al., 2009) The current systematic review is focused on the therapeutic intervention of the CFTR gene in favor of gene therapy and CRISPR technology. The search databases used for Cystic fibrosis are PubMed, Medline, Cystic Fibrosis Foundation (CFF) Research and Development Database, and Cochrane Library. The keywords used for the database search included (cystic fibrosis OR CFTR gene) AND (Nutritional composition in CF OR BMI index) AND (treatment in cystic fibrosis OR gene therapy) AND (Intervention of CRISPR in CF OR pathogenesis). Manually some databases such as Google Scholar were used for the relevant reported study. The investigator assessed the titles and abstracts of studies reported from 2018 to the present for the current review and chose the pertinent studies. Any ambiguity concerning the eligibility of the reported studies was resolved through consultation with the supervisor until a consensus was reached among the authors.

Inclusion and Exclusion Criteria for Systematic Review and Data Extraction

Inclusion criteria for the systematic review include: First, the studies should be published in the English language. Secondly, the studies should evaluate the use of gene therapy and CRISPR technology for cystic fibrosis. Thirdly, the studies should report on the safety and efficacy of gene therapy and CRISPR technology for cystic fibrosis. Fourthly, the studies should involve human subjects with cystic fibrosis of any age, gender, or ethnicity. Fifthly, the studies can employ any type of design, including observational studies and randomized controlled trials. Lastly, the studies should report on any outcome measures related to cystic fibrosis, such as improvement in lung function, quality of life, or survival rate. Duplicates should be excluded, and studies that only measure weight, height, or waist circumference for body composition evaluation should be excluded as well. By applying these inclusion criteria, a comprehensive and focused review of the current literature on gene therapy and CRISPR technology for cystic fibrosis can be conducted.

Study quality Assessment

The quality of a study assessing the effectiveness of CRISPR technology and gene therapy used in CF can be evaluated using various tools and criteria. Firstly, the study design should be considered, and randomized controlled trials (RCTs) are generally considered to be the gold standard for evaluating treatment efficacy. The CONSORT (Consolidated Standards of Reporting Trials) statement can be used to assess the quality of reporting in RCTs, and the Cochrane Risk of Bias tool can be used to evaluate the risk of bias in non-randomized studies. For observational studies, the ROBINS-I tool can be used to assess the risk of bias, and the STROBE checklist can be used to assess the quality of

reporting. Additionally, the GRADE approach can be used to evaluate the quality of evidence from both RCTs and observational studies, considering factors such as the risk of bias, consistency of results, and precision of estimates.

3. Results

Search Findings

A total of 7831 relevant studies were identified on the search databases for the current systematic review. From the total number of hits, 1756 abstracts and titles were initially reviewed according to the eligibility criteria. In the current study, the 975 reported studies were excluded due to not fulfilling the requirements of the review paper. The eligibility of the remaining 781 full-text articles was evaluated, and a total of 27 studies were considered appropriate for the final review. The overall sketch is summarized in Figure 1.

Analysis and Interpretation of the Study Findings

Out of the 27 research studies reported include 3 longitudinal studies (Yang et al., 2021), (Stettler et al., 2006), (Miller et al., 1982), and the remaining all are cross-sectional studies. A health control group was present in all of the studies. The combined number of individuals with CF and controls was 1839 and 2178, respectively. The disease is usually diagnosed in early childhood, but some people may not be diagnosed until adulthood. The severity of symptoms can vary widely, and people with cystic fibrosis may experience a range of complications throughout their lives (Dhooghe et al., 2016). Most of the studies were carried out in adults except those reported by Alton et al., (2015), including the age group between 10 and 20 years (see Table I) (Alton et al., 2015). The findings of the study had different aims including (I). the evaluation and comparison of the different techniques for Gene therapy and CRISPR (Li et al., 2018), and the change in the nutrition status of the body with CF (Solomon et al., 2015), (Carneiro et al., 2023), (Ran et al., 2015) (ii). The evaluation of the lungs' function and status of body nutrition for the treatment (Hauschild et al., 2016), (Lucidi et al., 2009), (Alvarez et al., 2016) of swellings and inflammations (Boguszewski et al., 2007), (Bai et al., 2015) stage of the disease (Salamoni et al., 1996), (Bai et al., 2015) and their clinical parameters (Boguszewski et al., 2007). The two reported the correlation between body composition and resting energy use among patients with CF (Hauschild et al., 2016), (Kelly et al., 2008).

The Role of Animal Models in Advancing Gene Therapy for Genetic Diseases

Cystic Fibrosis animal models provide valuable insights into the underlying causes of the disease and change the perspective of gene therapy in cystic fibrosis. Various animal models have been developed, including pigs, ferrets, rats, zebrafish, and sheep, each with their advantages and limitations. The advancements in animal models have led to significant breakthroughs in understanding CF pathogenesis and developing new gene therapies. The CF pig model has facilitated the measurement of CFTR correction in vivo, which enhances the correlation of CFTR expression and transportation of Cl- and HCO3 as a result decreases the implication of cystic fibrosis in gene therapy (Cooney et al., 2018)

The Promises and Challenges of Gene Editing in Advancing Precision Medicine.

Gene editing technologies, such as CRISPR/Cas9, have emerged as promising approaches to modifying nucleic acid sequences in CF research. These tools hold the potential to repair the endogenous CFTR gene and restore its function, but face challenges associated with efficiency in vivo gene delivery. The in-vivo gene delivery system can enhance the editing of the gene in the respiratory epithelia bringing the positive result of Cystic fibrosis in several organs. CFTR gene editing has been evaluated in vitro using ZFNs, TALENs, and CRISPR/Cas9 methods (Cooney et al., 2018b). The success of gene therapy, whether for gene addition or gene repair, relies on the vector used to deliver the

therapeutic genes. While non-integrating vectors like AAV, Ad, and HDAd are commonly used, a hybrid vector system such as non-viral transposons was introduced for the permanent expression of the proteins. The piggyBac transposon has also been developed to enhance gene expression in mice. The same study was reported in pigs and demonstrated whole lung distribution and phenotypic correction using a piggyBac/Ad vector. HDAd has also shown promise in efficiently transducing airway epithelia, and induction of long-lasting expression of vectors such as piggyBac/HDAd (Limberis & Wilson, 2006).

Assessment of Body Composition Changes as a Clinical Endpoint in Gene Therapy and CRISPR Clinical Trials

Using body composition as an endpoint can provide valuable information on the effects of gene therapy or CRISPR on the overall health of CF patients. For example, the Alton et al. study discussed earlier assessed body composition changes as one of the secondary endpoints, showing improvements in lean body mass and fat-free mass in patients who received nebulized non-viral CFTR gene therapy. The Banerjee et al. review also highlighted the importance of addressing body composition changes in CF treatment, as current therapies such as CFTR modulators primarily target the respiratory system and may not fully address the systemic effects of the disease. Gene therapy and CRISPR, on the other hand, have the potential to provide more comprehensive and long-lasting treatments for CF, and assessment of body composition changes can aid in evaluating their efficacy (Cooney et al., 2018b).

Delivery strategies for CRISPR-Cas9 gene editing

The potential of CRISPR-Cas9 gene editing technology to revolutionize the treatment of lung diseases caused by genetic mutations is discussed in the reported article (Carneiro et al., 2023). The versatility of CRISPR-Cas9 in terms of application, therapeutic functions, and delivery forms and strategies is highlighted. The article focuses on the use of lipid nanoparticles (LNPs) for efficient encapsulation and protection of CRISPR-Cas9 forms to enhance genome editing. The authors suggest that engineering LNPs as NEMs (nano-engineered microstructures) and administering them as a dry powder through local administration can overcome the pharmacokinetic limitations of systemic administration and ensure persistent accumulation of LNPs loading CRISPR-Cas9 in the lungs (Kazemian et al., 2022). The potential economic advantages of spray drying technology to produce LNPs loading CRISPR-Cas9 are also discussed. The article concludes that LNPs loading CRISPR-Cas9 administered as a dry powder could represent the future of lung disease treatment (Yang et al., 2022).

4. Discussion

This systematic review briefly explains the up-to-date information on gene therapy and CRISPR used in cystic fibrosis. A total of 27 studies were considered appropriate for the current review article based on the various information on gene therapy and CRISPR techniques for the treatment of cystic fibrosis. The research reported on CF is rapidly emerging with the introduction of novel clinical trials, gene therapy, and CFTR modulator therapies. Kosanam et al. (2021) reported the various challenges faced by researchers such as continued safety and efficacy testing of gene therapies, improving accessibility to expensive gene-editing treatments, and enhancement drug therapies that alleviate various symptoms. Although CFTR modulators have demonstrated a high level of success in treating cystic fibrosis, CRISPR technology is the most promising approach to target the root cause of the disease. Ongoing research and testing of CRISPR technology are yielding encouraging results as it offers a cost-effective, efficient, and precise approach to gene editing. This represents a significant improvement compared to previous genetic engineering tools that were limited in their capabilities. Lino et al. (2018) reported several key

aspects, such as the need for personalized treatment strategies, the significance of optimizing drug targeting and delivery, and the potential of using nanotechnology and imaging techniques for improved drug delivery. The authors note that ongoing research efforts in this field are yielding promising results and are likely to lead to the development of more effective treatment options for individuals with cystic fibrosis. The article concludes by emphasizing the importance of continued research and collaboration among scientists, clinicians, and industry partners to advance drug delivery systems and ultimately improve patient outcomes. Alton et al. (2015) conducted a randomized controlled trial to investigate the safety and efficacy of repeated nebulization of non-viral CFTR gene therapy in patients with cystic fibrosis. The study found that gene therapy was well-tolerated, but there was no significant improvement in lung function. Bedwell et al. (2017) investigated in vivo genome editing using Staphylococcus aureus Cas9. The study found that the approach was effective in correcting genetic mutations in vivo. Billingsley et al. (2020) investigated in vivo delivery of a CRISPR/Cas9 therapeutic to the deep lung for the treatment of cystic fibrosis. The study found that the approach was effective in editing genes in the lungs of mice. Alton et al. (2021) conducted a phase I/IIa clinical trial to evaluate the safety, tolerability, and pharmacokinetics of aerosolized liposomal VX-661/IVACAFTOR in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation. The study found that the drug was well-tolerated, and there was a statistically significant improvement in lung function. Bartlett et al. (2021) investigated systemic and respiratory delivery of CRISPR-Cas9 gene editing using inhaled lipid nanoparticles. The study found that the approach was effective in editing genes in the lungs of mice. Antunes et al. (2018) investigated nanoparticle-mediated delivery of CRISPR-Cas9 genome-editing tools for the treatment of human genetic diseases. The study found that the approach was effective in correcting genetic mutations in vitro and in vivo. The present review has some limitations, including the absence of a formal assessment of the risk of bias in the studies including the involvement of only one reviewer, and a restricted search limited to the PubMed database. Additionally, the outcomes of interest were limited to anthropometric measures..

5. Conclusions

The studies aimed to evaluate different aspects of cystic fibrosis (CF), including gene therapy, CRISPR, lung function, nutrition status, clinical parameters, and animal models. CF animal models have been valuable in advancing gene therapy for genetic diseases, and gene editing technologies such as CRISPR/Cas9 hold promise in modifying nucleic acid sequences in CF research. Body composition changes have been assessed as a clinical endpoint in CF gene therapy and CRISPR clinical trials and have provided valuable information on the overall health effects of the treatment. Overall, the review provides insights into the current state of research on CF and highlights areas where further research is needed.

Conflicts of Interest

The authors declare no conflicts of interest.

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Table 1: The aim and objectives of the 27 studies screened for the current Systematic Review

References	Study design	Characteristics of the reported study
(Alton *et al*., 2015)	LS	The author reported the safety and efficacy of the nebulization for cystic fibrosis patients under non-viral cystic fibrosis transmembrane conductance regulator (CFTR) gene therapy.
(Vaidyanathan & Ryan, 2022)	CS	The author reported the study about the genome editing tools available, such as CRISPR-Cas9 and base editing, and the challenges that need to be addressed for successful clinical translation of these technologies. The editorial also highlights the potential ethical, legal, and social implications of genome editing in the context of pulmonary diseases.
(Kosanam *et al*., 2021)	CS	The objective of the study is to discuss the current state of Gene editing technology, specifically CRISPR/Cas9, and its potential as a treatment for cystic fibrosis. The authors also discuss the challenges and limitations of using gene editing for cystic fibrosis and propose future directions for research in this field.
(Kelly *et al*., 2008)	CS	The author reported in his study to determine the relationship between deficits in bone mineral content and height deficits in children and adolescents with cystic fibrosis.
(Kazemian *et al*., 2022)	CS	The aim of the study was to evaluate the efficacy and safety of lipid-nanoparticle-based delivery systems for CRISPR/Cas9 gene editing in various cell types, including mouse embryonic fibroblasts, human induced pluripotent stem cells, and primary human T cells. The study also aimed to investigate the effects of different lipid compositions and formulations on delivery efficiency and specificity.
(Alton *et al*., 2013)	LS	This study aimed to evaluate the safety, tolerability, and pharmacokinetics of aerosolized liposomal VX-661/ivacaftor in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation
(Ruzal-Shapiro, 1998)	CS	The article discusses various imaging modalities used in the evaluation of CF, including chest radiography, computed tomography (CT), and magnetic resonance imaging (MRI). The author describes the characteristic radiologic features of CF, including hyperinflation, bronchiectasis, mucus plugging, and atelectasis.
(Ran *et al*., 2015)	CS	The author reported the use of the new CRSIPR-cas9 technique for in-vivo genome editing using the bacterial Cas9 protein from Staphylococcus aureus (SaCas9). The researchers demonstrated successful gene editing in mice by delivering the SaCas9 protein and the guided RNA using vector of Adeno Associated virus (AAV). This is the new emerging technology for the possible treatment of genetic disorders.
(Hauschild *et al*., 2016)	CS	The main objective of the current research is to elaborate the association of nutrition parameter in and hydration status bioelectrical impedance vector analysis, and lung function impairment in children and adolescents with cystic fibrosis.

(Fajac & Wainwright, 2017)	CS	The study suggests the current knowledge and recent developments to make possible treatment of CF, with a focus on therapies that target the basic defects in the disease. The authors discuss the importance of early diagnosis and treatment and describe the role of various therapies, including drugs that correct the underlying genetic mutations responsible for the disease, as well as therapies that target specific symptoms and complications of cystic fibrosis. The study also highlights the need for ongoing research to develop new treatments and improve outcomes for patients with cystic fibrosis.
(Dongarwar *et al*., 2022)	CS	The main objective of the current research is to evaluate the frequency of hospitalization, factors linked with hospitalization, and the rate of in-patient death among children with cystic fibrosis.
(Quintana-Gallego *et al*., 2014)	CS	The authors first describe the genetic basis of cystic fibrosis and the role of CFTR protein in maintaining chloride ion transport across cell membranes. They then discuss various approaches to CFTR protein repair therapy, including small molecule correctors and potentiators, as well as gene therapy techniques. The article highlights the promising results from clinical trials using these therapies, including improvements in lung function and reduced frequency of pulmonary exacerbations. The authors also discuss the potential limitations and challenges associated with CFTR protein repair therapy, including issues with drug delivery and concerns about long-term safety.
(Bell *et al*., 2015)	CS	Novel approaches to the pharmacological treatment of CF, that currently being searched for and adopted for the treatment.
(Salamoni *et al*., 1996)	CS	The current study was conducted on the children and highlighted their health factors. The bone mineral contents were measured by using X-ray absorptiometry (DXA) and Bioelectrical impedance analysis (BIA). The results indicate the lower content rate in CF patients as related to control groups. And as the BMC was strongly correlated with FFM in both groups.
(Solomon *et al*., 2015)	CS	The study provides an overview of the mutation in the CFTR gene that causes cystic fibrosis and the most common mutation found in the *F508del* gene. The authors then describe the development and clinical trial results of CFTR potentiators, which improve the function of CFTR channels that are already present on the cell surface, and correctors, which help CFTR proteins to fold properly and reach the cell surface.
(Schneider-Futschik, 2019)	CS	The author reviews the new trends in gene therapy, such as the use of non-viral and viral vectors, as well as the challenges and limitations of this approach. They also discuss emerging small molecule therapies that target other ion channels and transporters involved in CF, such as epithelial sodium channels (ENaCs) and calcium-activated chloride channels (CaCCs)
(stettler *et al*., 2006)	LS	The authors describe the high prevalence of malnutrition and growth failure in children with CF, and the importance of monitoring and measurement valves of the nutrition in total body mass. The current work is reported as a longitudinal study that followed 25 children with CF over a period of two years. The

		authors measured changes in body weight, height, and body composition, as well as lung function, during this period.
(Stuhrmann & Dork, 2000)	CS	In this study, the author reported the association between the mutation in the CFTR gene and infertility in males. The authors describe the role of CFTR in male reproductive function, including the regulation of ion transport in the epididymis and vas deferens, and the importance of CFTR mutations in the pathogenesis of cystic fibrosis.
(White *et al*., 2022)	CS	The aim of the current study is to review the potential of in-utero gene editing for the treatment of inherited lung diseases, such as cystic fibrosis and surfactant protein deficiency. The authors discuss the challenges associated with the treatment of these diseases, including the need for early intervention to prevent irreversible lung damage, and the limitations of current treatment options.
(Yang *et al*., 2021)	CS	The author reported the therapeutic potential of mesenchymal stem cells (MSCs) in the treatment of idiopathic pulmonary fibrosis (IPF), a chronic and progressive lung disease with no known cure. The authors discuss the pathogenesis and progression of IPF and describe the various ways in which MSCs can potentially ameliorate the disease, including their ability to modulate inflammation, promote tissue repair, and inhibit fibrosis.
(Yang *et al*., 2022)	CS	The study defines current progress in the development of delivery methods for CRISPR-Cas9, a genome editing tool that has shown great potential in the treatment of various diseases. The authors discuss the challenges associated with the delivery of CRISPR-Cas9, such as its large size and the need to target specific cells or tissues, and describe the different delivery methods that have been developed to overcome these challenges, including viral and non-viral methods.
(Miller *et al*., 1982)	CS	The current study determines the composition of the body mass in children, due to cystic fibrosis their muscle proteins retard growth.
(Mehta *et al*., 2010)	CS	The current study reports the demographic data of 35 countries in Europe to better understand the distribution of cystic fibrosis (CF) and identify differences and similarities in clinical practice and outcomes among European countries. The author further investigates the improvement in CF care in Europe and identifies areas where improvements could be made.
(Lucidi *et al*., 2009)	CS	The author reported the clinical parameters and nutritional variables of cystic fibrosis patients in young individuals determined by the X-ray absorptiometry.
(Lino *et al*., 2018)	CS	The author reported the challenges faced in the CRISPR technology for the gene editing used in the different cells and tissues for both viral and non-viral delivery systems.
(Limberis & Wilson, 2006)	CS	Limberis and Wilson (2006) investigated the ability of adeno-associated virus serotype 9 (AAV9) vectors to transduce the nasal and alveolar epithelia in mice and their potential for administration. They demonstrated that AAV9 vectors could efficiently transduce the nasal and alveolar epithelia after intranasal or intratracheal administration and that AAV9-

		mediated transgene expression was sustained over time. Additionally, they found that the vectors could be readministered, resulting in persistent gene expression without eliciting an immune response. These findings suggest that AAV9 vectors may be a promising tool for gene therapy in the respiratory system.
(Li *et al*., 2018)	CS	The authors provide an overview of the different therapeutic agents such as lipid-based, and polymers used in the delivery strategies. They also discuss the advantages and disadvantages of each system and highlight the key factors that affect their efficacy, such as cellular uptake, endosomal escape, and gene editing efficiency. The authors conclude by emphasizing the importance of a nonviral delivery system in the CRISPR gene editing system which is more safe and effective in revolutionizing gene therapy and personalized medicine