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Gene Therapy for Cystic Fibrosis: Overcoming Current Limitations and Future Directions

Nadir Ali Wassan[1], Asfia Qammar[2], Shahid Burki[3], Anam Arshad[4], Hassam Gul[5], Muhammad Mujtaba[6]

[1]Jinnah Postgraduate Medical Center, Karachi, Sindh, Pakistan.
[2]Dow Medical College, Karachi, Sindh, Pakistan.
[3]Allama Iqbal Medical College, Lahore, Punjab, Pakistan.
[4]Department of Biochemistry, Central Park Medical College, Lahore, Punjab, Pakistan.
[5]International Islamic University, Islamabad, Pakistan.
[6]School of Chemistry, University of the Punjab, New Campus, Lahore, Punjab, Pakistan.

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Corresponding Author: Nadir Ali Wassan,
Jinnah Postgraduate Medical Center, Karachi, Sindh, Pakistan.
Email: wassannadir110@gmail.com

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ABSTRACT

Cystic fibrosis (CF) is a lethal inherited disorder resulting from mutations in the CFTR gene that cause improper chloride ion movement and clogging of the lungs with mucus. While classic therapies have been augmented by CFTR modulators and airway clearance techniques, these remain limited by expense, specificity for the mutation, and the need for lifetime therapy. Gene therapy has been suggested as a curative treatment, wherein the goal is to repair the defective CFTR gene through techniques such as CRISPR-Cas9, viral/non-viral vectors, and stem cell-based therapy. This study employed a mixed-methods setting, integrating survey-based assessment with a meta-analysis of existing literature to evaluate awareness, perception, and barriers to implementation of gene therapy among CF patients and caregivers in Punjab, Pakistan. A questionnaire was given to 65 participants, and qualitative data analysis with SPSS established a significant awareness gap, where socioeconomic status and education level were major determinants. Chi-square and ANOVA tests established statistically significant relationships between demographic variables and gene therapy acceptance. Moreover, the meta-analysis found consistent trends in current research indicating similar problems of limited accessibility, ethical concerns, and the necessity for improved gene delivery systems. The results reaffirm the necessity for further patient education, increased affordability, and more clinical verification to make gene therapy a therapeutic reality. In general, the research underscores the promise of gene therapy in CF while encouraging the implementation of multidisciplinary methods to overcome existing limitations. This study examines awareness and perceptions of gene therapy for cystic fibrosis in Punjab, Pakistan, revealing low knowledge levels influenced by socioeconomic factors. Challenges include cost, accessibility, safety concerns, and ethical implications. Future efforts must integrate research, policy reforms, and patient advocacy to enhance gene therapy's feasibility and availability.

INTRODUCTION

Cystic fibrosis (CF) is a fatal genetic condition that arises as a consequence of CFTR gene mutations, coding for a chloride channel that manages the movement of salt and water through epithelial membranes (Ratjen & Döring, 2003). CFTR mutations lead to abnormal chloride ion transport that culminates in the build-up of thick and sticky mucus in lungs, pancreas, and other organs. The perturbed mucosal environment predisposes patients to frequent infections, inflammation, and relentless lung devastation that are the leading determinants of morbidity and mortality among CF patients [1]. Conventional therapy strategies focus on symptom suppression and dampening the progression of disease but lack addressing the pathogenetic genetic flaw, hence calling for exploring curative methods such as gene therapy [2].

Gene therapy is aimed at fixing the defective CFTR gene or providing functional copies to restore normal chloride ion transport, thus offering a cure for cystic fibrosis (CF). The past decades have seen phenomenal progress in the creation of gene delivery systems, including viral and non-viral vectors, which enable the delivery of therapeutic genes into airway epithelial cells [3]. However, several obstacles have limited the clinical

effectiveness of gene therapy for CF, including poor gene transfer, immune reactions, and failure to achieve long-term CFTR expression in lung tissues [4]. Moreover, the dense mucus layer and inflammation found in CF lungs present additional challenges to gene delivery, thus limiting the effectiveness of existing methodologies [5].

In spite of these challenges, new trends in molecular biology and genetic engineering have resulted in new approaches to optimize gene therapy efficiency. New genome editing tools like CRISPR-Cas9, better viral vectors like lentiviruses and adeno-associated viruses (AAVs), and non-viral nanoparticle-based platforms present new directions for the potential bypassing of current limitations [6]. Additionally, the recent advances in stem cell therapy and personalized medicine strategies have the ability to revolutionize CF therapy by allowing targeted correction of patient-specific mutations [7].

Limitations of Current Treatments

The traditional treatments for cystic fibrosis (CF) mainly aim to treat symptoms instead of the actual genetic defect. Some of the therapies that are available at present are airway clearance techniques, mucolytics, antibiotics, and CFTR modulators, and they are designed to alleviate respiratory symptoms, lower the infection rates, and enhance lung function as a whole [8]. These therapies have greatly improved the quality of life and increased the life expectancy of CF patients in the last few decades. They do, however, need strict, lifelong compliance and do not provide a cure, which is a heavy burden on both patients and healthcare systems [9].

Although progress has been made in CF management, current therapies are not all effective. CFTR modulators, including Ivacaftor and the triple combination Elexacaftor-Tezacaftor-Ivacaftor, have been able to restore a degree of CFTR function in patients with particular mutations, including the prevalent Phe508del mutation [10]. These treatments are, however, mutation-specific and do not benefit patients with uncommon or nonsense mutations, and therefore a group of CF patients do not have a targeted therapy available. Furthermore, the expense of CFTR modulators is high, making it inaccessible in low-resource environments, further highlighting the necessity for a more universally effective treatment approach [11, 12].

Another significant drawback of existing CF therapies is that they cannot stop the progression of disease altogether. Antibiotics and anti-inflammatory drugs that treat lung infection and inflammation may alleviate symptoms, but they fail to stop progressive loss of lung function over the long term [13]. Even with maximally optimized treatment strategies, CF patients still suffer from exacerbations and permanent lung injury. As these obstacles, researchers have come to realize the importance of seeking an advanced therapy directed at addressing the fundamental basis of CF on the genetic level. Gene therapy can offer the ultimate solution through directly correcting or substituting the aberrant CFTR gene and ultimately providing a sustained or curative therapy for every CF patient across all mutation categories [14].

Gene Therapy as a Potential Cure

Gene therapy is a potentially valuable option in the management of cystic fibrosis (CF) in that it corrects the cause of the condition rather than solely the symptoms. Gene therapy primarily seeks to add a functional CFTR gene into cells that have the defect, with the purpose of correcting the defective chloride ion transport and enhancing clearance of the mucus [15]. Several approaches have been tried to accomplish this, ranging from viral and non-viral vectors for gene transfer and genome-editing systems like CRISPR-Cas9. Stem cell-based therapeutic strategies have also been considered as a way of recreating lung tissue containing functional CFTR-expressing cells [16]. Although initial studies have shown the promise of these methods, their translation into viable and long-term treatments continues to pose a major obstacle.

One of the primary challenges for effective CF gene therapy is effective gene delivery. The dense mucus layer within CF lungs also serves as a physical barrier to therapeutic vectors penetrating target cells. Viral vectors, including adeno-associated viruses (AAVs) and lentiviruses, have proven to be beneficial for gene delivery but are also limited by, for example, limited packaging capacity and possible activation of the immune system [8, 17]. Non-viral delivery platforms, including electroporation and lipid nanoparticles, offer immune reduction alternatives at the cost of poorer gene transfer efficacy. Furthermore, even when there is successful delivery, long-term CFTR expression is still an obstacle since epithelial cells lining the lung tend to have a high turnover naturally, which will result in cell loss of the corrected cells as well as a requirement for periodic treatments [18].

In addition to delivery issues, gene therapy for CF also has to overcome immune-related hurdles and safety issues. The immune system can identify viral vectors as foreign and respond with an immune reaction, diminishing the efficacy of treatment and elevating the risk of inflammation [19]. Furthermore, genome-editing methods like CRISPR-Cas9, although extremely specific, carry risks of off-target genetic alterations that may result in unforeseen effects. Continued work is focused on optimizing these methods, enhancing vector design, and investigating new delivery systems that offer increased efficiency while reducing risk. As gene therapy continues to progress, these emerging methods have considerable promise for bringing a long-term or

even therapeutic intervention for CF, and for the first time, patients are offered new hope since they lack many current options [20].

Key Challenges in Gene Therapy for CF

Despite its potential, gene therapy faces several limitations that must be addressed for clinical success:

Inefficient Gene Delivery

Delivery of therapeutic genes into lung epithelial cells is a major challenge in gene therapy for cystic fibrosis (CF) because of the intricate biological milieu of the respiratory tract. One of the main challenges is the thick, sticky mucus that defines CF airways. The excess mucus buildup creates a thick barrier that prevents penetration by gene delivery vectors, making them less efficient at reaching target epithelial cells [21]. The mucus barrier is not only a physical hindrance but also has antimicrobial peptides and oxidative agents present, which can break down viral and non-viral vectors, making delivery even less efficient. Therefore, the formulation of methods to penetrate or thin the mucus layer, including the use of mucolytic drugs or specially designed nanoparticles, is essential for improving gene delivery in CF patients [16, 22].

In addition to the mucus seal, chronic inflammation of CF lungs further hinders gene delivery. Continuous bacterium infection and immune response trigger neutrophil recruitment and secretion of inflammatory cytokines, creating a deleterious environment for gene delivery vectors [23]. The heightened immune response not only damages the airway epithelium but also impinges on the viability of gene therapy vectors by triggering their clearance from the lung. In addition, inflammatory processes can modify epithelial cell surface receptors, which are usually required for vector binding and entry, further inhibiting gene transfer efficiency. Production of anti-inflammatory drugs along with gene delivery systems can increase the effectiveness of gene therapy by allowing a more favorable condition for the uptake of vectors [24].

High cell turnover in the respiratory epithelium is another obstacle to long-term gene expression. Epithelial cells lining the lungs are continuously lost and replaced as a result of normal cell turnover and injury from chronic inflammation, so any corrected cells are rapidly lost and replaced by uncorrected cells [25]. Such high turnover requires multiple administrations of gene therapy, making treatment more complex, expensive, and risky [12]. Scientists are researching long-term gene expression approaches, including the integration of therapeutic genes into the host genome or employing stem cell-based therapies to create a population of self-renewing corrected cells. Overcoming these delivery challenges is critical for achieving long-term gene expression and optimizing the therapeutic potential of gene therapy for CF [26].

Short-lived Gene Expression

One of the most challenging issues in cystic fibrosis (CF) gene therapy is sustaining long-term expression of the CFTR gene in lung tissue. Numerous gene therapy approaches present promising initial efficacy, yet they tend to have short-term duration and must be repeatedly administered in order to preserve therapeutic effects [27]. This transient gene expression is mostly because of the failure of existing gene delivery vectors to integrate the therapeutic gene into the host genome for stable, long-term expression. The majority of viral vectors employed in CF gene therapy, including adeno-associated viruses (AAVs), introduce the CFTR gene as an episome—an extrachromosomal segment of DNA that is not integrated into the host cell's genome. Although episomal expression is able to restore CFTR function transiently, it decreases with time as cells proliferate and substitute the therapeutic gene with newly synthesized, uncorrected DNA [28]. This reduction in therapeutic gene copies restricts the duration of gene expression, requiring repeated administration and complicating treatment [29].

Another major reason for transient gene expression is the rapid turnover of lung epithelial cells. The respiratory epithelium is constantly being regenerated, especially in CF patients, where chronic infection and inflammation enhance cellular turnover [30]. As corrected cells are lost and replaced by newly formed, uncorrected cells, the therapeutic effect of gene therapy slowly fades away. This fast rate of cell turnover implies that even if a successful gene transfer is achieved, its effect is temporary and requires repeated gene therapy doses in order to maintain clinical effects. Furthermore, repeated dosing involves other issues, including the potential for immune reactions against viral vectors. When the immune system of a patient becomes aware of the vector after treatments, later injections could lead to immune responses that neutralize the vector before it is able to provide its genetic payload, thereby lessening its efficiency [31].Research continues to address the limitation of transient gene expression, and scientists are finding new ways to ensure sustained CFTR gene expression. A possible solution is inserting therapeutic genes into the host genome through lentiviral or gene-editing technologies such as CRISPR-Cas9, which might lead to long-term correction of CFTR mutations [32]. Further, stem cell-based therapies promise long-term answers through the infusion of genetically corrected progenitor cells that continue to produce corrected epithelial cells. Other studies are centered on the optimization of non-viral delivery vectors, e.g., lipid nanoparticles, in a manner that makes repeated injections feasible without evoking potent immune responses [33]. Although these methods are still in the process of being studied, their effectiveness may lead to breakthrough CF

gene therapy with less frequent administration and a more long-lasting therapeutic effect [34].

RESEARCH OBJECTIVES

1. To assess the efficacy of gene therapy strategies in the restoration of CFTR function based on survey analysis and meta-analysis of clinical and preclinical data.
2. To determine the major limitations of existing gene delivery strategies, such as inefficient transfer of genes, immune reactions, and transient expression of genes, through a review of the literature and synthesis of data.
3. To investigate new strategies, including CRISPR-Cas9 genome editing, enhanced viral/non-viral vectors, and stem cell therapies, for circumventing current limitations in CF gene therapy.

Problem Statement

Cystic fibrosis (CF) is a potentially lethal genetic illness due to mutations in the CFTR gene that result in aberrant chloride ion transport, thickened mucus accumulation, and irreversible lung injury. Although existing treatments, i.e., CFTR modulators and symptomatic drugs, have enhanced patient prognosis, they are not permanent cures and are ineffective for those with uncommon mutations. Gene therapy offers a potential answer by directly addressing the genetic defect, but limitations like ineffective gene delivery, transient gene expression, and immune reactions have prevented its universal success. Based on these shortcomings, there is an urgent necessity to assess the state of CF gene therapy today, uncover existing hindrances, and investigate novel approaches to maximize its potential.

Significance of the Study

This research is important as it gives a comprehensive overview of CF gene therapy's progress and problems, providing helpful information to researchers, clinicians, and policymakers. Through the conduct of survey analysis and meta-analysis, the research will lead to a better comprehension of the effectiveness of current gene therapy strategies and the main restrictions that need to be resolved. In addition, by investigating new tactics like CRISPR-based gene editing, better viral and non-viral vectors, and cell therapies, this research seeks to identify possible breaks that may help develop more impactful and sustained remedies for CF. Eventually, such research may find its way towards informing future developments in therapy, enhancing patient performance, and progressing precision medicine against CF.

LITERATURE REVIEW

Current Treatment Approaches for Cystic Fibrosis

Cystic fibrosis (CF) is a fatal hereditary disease caused by mutations within the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene, resulting in impaired chloride ion transport and mucostasis within the lungs, pancreas, and other tissues [35]. Numerous improvements have occurred in the treatment of CF in recent years with a major emphasis on symptom suppression, infection control, and arresting disease progression. The conventional therapies comprise airway clearance methods like chest physiotherapy and high-frequency chest wall oscillation, which aid in mucus mobilization and clearance from the airways and minimize infection risk [36]. Moreover, mucolytic therapy such as dornase alfa and hypertonic saline thin the mucus to facilitate easy expulsion. These treatments, with bronchodilators and anti-inflammatory drugs, have enhanced the lung function and quality of life in most patients with CF but do not remove the genetic flaw, requiring compliance for a lifetime and ongoing medical interventions.

A significant advancement in CF therapy has come with the establishment of CFTR modulators, which act on the mutant CFTR protein to correct its function. Ivacaftor, the initial certified CFTR modulator, is a potentiator that increases CFTR channel activity in individuals harboring gating mutations, including G551D [37]. Following developments resulted in combination therapies such as Lumacaftor-Ivacaftor and Tezacaftor-Ivacaftor, which enhance protein folding and cell membrane trafficking in individuals with the prevalent F508del mutation [38]. The greatest progress so far is the triple combination treatment Elexacaftor-Tezacaftor-Ivacaftor, which gives extensive enhancements in lung function and symptom relief in a wider population of CF patients. Nevertheless, though very effective, CFTR modulators are mutation-specific and are ineffective for patients who have rare or nonsense mutations, hence their utility to the overall CF population is restricted. Also, due to their high price, they are out of reach to many patients all over the world, presenting economic and ethical problems in managing CF [39].

Despite these developments, current CF treatment does not ensure a lasting cure since it only manages symptoms rather than the fundamental genetic defect. Anti-inflammatory therapy and antibiotics are usually used to manage recurrent infections by bacteria and reduce inflammation within the airways but cannot prevent long-term deterioration of the condition [40]. Lung transplant remains an option for end-stage CF patients but is fraught with risks such as organ rejection and lifelong immunosuppression. The limitations in the available therapy make alternative therapy strategies an imperative need, such as gene therapy, which promises to correct the defective CFTR gene at the source and provide a long-term or curative treatment for all CF patients.

Gene therapy presents a groundbreaking strategy for the treatment of cystic fibrosis (CF) by addressing the

very cause of the disease—the mutated CFTR gene. In contrast to traditional treatments, which only deal with symptoms, gene therapy seeks to restore chloride ion transport through the introduction of a healthy CFTR copy into lung epithelial cells, improving mucus clearance and slowing down disease progression [41]. The most widely investigated approach is through viral vectors, specifically adeno-associated viruses (AAVs) and lentiviruses, and these have been successful in the delivery of the CFTR gene to the target cells. Research by [42] showed that AAV-mediated gene transfer had the ability to restore CFTR activity in animal models, giving a proof of principle for its use in a clinical context. Though these strategies have good prospects, they come with great challenges like immune response against viral vectors, which can decrease their efficiency and limit the possibility of repeated dosing [43]. Early clinical trials with AAV-based vectors also resulted in transient outcomes, indicating the necessity for efficient delivery mechanisms with sustained gene expression.

As a countermeasure to the shortcomings of viral vectors, scientists have turned to non-viral gene delivery systems, including lipid nanoparticles, electroporation, and polymer-based systems. Non-viral approaches have the benefits of lower immunogenicity and the capacity to carry larger genetic loads [44]. Nevertheless, these systems are plagued by low transfection efficiency, where few lung cells are able to uptake the therapeutic gene. [45] examined lipid nanoparticle-mediated delivery of CFTR in CF models and concluded that, although the method was well tolerated, gene transfer efficiency was much lower than with viral vectors. A further study by [46] proposed that a combination of non-viral vectors with airway surface modifiers, including mucolytic agents, might enhance gene uptake by diminishing mucus barriers. In spite of these developments, additional optimization is needed to maximize gene transfer efficiency and achieve long-term therapeutic outcomes.

More recently, a breakthrough in CF gene therapy is the use of genome-editing technologies such as CRISPR-Cas9, which directly edit mutations at the DNA level, possibly providing a permanent cure [47]. Unlike the conventional gene replacement methods, CRISPR enables direct editing of the faulty CFTR gene to its normal function without requiring repeated doses. Research by [48] has demonstrated that CRISPR-mediated repair of CFTR mutations in patient-derived epithelial cells effectively restored chloride ion transport. Nevertheless, issues of off-target effects, delivery efficiency, and the possibility of unwanted mutations are still significant barriers to clinical translation. Current research is aimed at enhancing the specificity of CRISPR-based therapies and the development of more efficient delivery systems, including nanoparticle-based CRISPR delivery or viral vector-mediated genome editing. While CF gene therapy is currently in the development phase, ongoing improvements in vector design, delivery systems, and genome editing tools hold promises for a sustained or curative therapy for patients with CF.

Challenges in CF Gene Therapy

Despite encouraging advances, there are a number of roadblocks to successful gene therapy for CF. Perhaps the biggest obstacle is inefficient delivery of genes. The dense mucus layer in the lungs of a person with CF functions as a physical obstacle, impeding gene therapy vectors from effectively reaching target epithelial cells [49]. Also, chronic airway inflammation in CF causes an immune response that degrades viral vectors prior to effective gene transfer [50]. A second principal limitation is the transient nature of gene expression. Epithelial cells in the lung turn over relatively quickly, such that corrected cells are often shed and replaced by uncorrected cells, requiring multiple gene therapy treatments [51]. Additionally, immune-mediated issues, including the immune response against viral vectors, constitute risks of inflammation and decreased efficiency in repeated administration [52]. These limitations emphasize the call for further research on maximizing gene delivery, enhancing transgene stability, and devising immune-evasive strategies.

Emerging Strategies to Overcome Gene Therapy Limitations

One of the most important challenges in CF gene therapy is effective gene delivery, as natural barriers within the lungs, including thick mucus and immune reactions, prevent the uptake of therapeutic genes. To address this, scientists are developing next-generation viral vectors with improved lung epithelial cell tropism and decreased immunogenicity. For instance, modified adeno-associated virus (AAV) variants with enhanced targeting have shown enhanced transduction efficiency and sustained gene expression in preclinical models [53]. Moreover, alterations in the structure of the viral capsid are being investigated to avoid immune recognition, enabling repeated dosing without inducing an unfavorable immune response. Lentiviral vectors with self-inactivating features have also been studied in recent research, which increases safety without compromising stable gene expression [54]. Such vector design advancements are essential for obtaining efficient and long-term gene expression in CF patients.

The other hope for CF gene therapy improvement is the incorporation of hybrid delivery systems, which involve the combining of viral and non-viral systems to achieve maximum safety and efficacy. Lipid nanoparticles (LNPs) are being explored as powerful delivery vehicles that can deliver gene-editing elements, including CRISPR-Cas9, to lung epithelial cells specifically. Research by [55] has established that the encapsulation of CRISPR components within LNPs makes them stable and allows accurate genetic

alterations with minimal immune activation. Biodegradable polymer nanoparticles are also in the process of being developed as alternatives to viral vectors, and these provide large cargo capacity at low immune-related side effects [56]. By combining these technologies with airway surface modifiers, including mucolytic agents or penetration enhancers, gene transfer is further enhanced by lowering mucus barriers in the CF lung. Such hybrid strategies represent an optimistic advance toward overcoming inefficiencies of conventional gene therapy methods.

Stem cell-based therapies are yet another novel approach to long-term correction of CFTR mutations in CF patients. In contrast to conventional gene therapy, which is needed to be administered repeatedly, stem cell therapy is designed to deliver a stable source of CFTR-expressing cells to the lungs. Scientists have used genetically engineered progenitor cells that can engraft into lung tissues and steadily produce functional CFTR-expressing epithelial cells [57]. Further, advances in induced pluripotent stem cell (iPSC) technology in recent years have made it possible to generate patient-specific stem cells that may be edited using CRISPR-Cas9 and transplanted with minimal chance of immune rejection [58]. Though at very early research phases, stem cell therapy is the potential means by which CF might be given a long-term or even a lifetime cure through fixing the disease at a cellular level. As these new strategies evolve, there is hope of breaking through the existing limitations of CF gene therapy and enhancing therapeutic outcomes for every patient [59].

METHODOLOGY

A cross-sectional survey design was used to evaluate the awareness, perceptions, and issues regarding gene therapy among CF patients and their caregivers in Punjab, Pakistan. Information was gathered from 65 participants, comprising CF patients and their main caregivers, from various hospitals in Punjab. A representative sample of people from various socio-economic and geographical locations was ensured. The hospitals that were chosen for the study were tertiary care facilities, pulmonology clinics, and specialty CF clinics. These hospitals were selected because they were accessible to CF patients and played an important role in the diagnosis and treatment of CF.

A purposive sampling method was employed to enroll participants, with only those having direct experience with CF being included in the study. The sample included clinically diagnosed CF patients (children and adults) and primary caregivers (parents, guardians, or close family members who take care of the patient). The participant inclusion criteria necessitated that the participants should be residents of Punjab, Pakistan, have a hospital-confirmed or self-reported diagnosis of CF, and agree to take part in the study. Healthcare professionals and researchers who took part as participants were excluded since the interest was in getting the views of patients and caregivers.

Data were gathered through a self-administered questionnaire to enable participants to respond to the survey on their own with minimal involvement of the researcher. The questionnaire was prepared in English and Urdu to cater to different literacy levels and contained both closed-ended and open-ended questions. It addressed important areas including demographics (age, sex, education, socioeconomic status, geographic region), gene therapy awareness (knowledge of CF genetics and acquaintance with gene therapy), treatment experience (difficulty with CF management and attitudes toward effectiveness of treatment), willingness to embrace gene therapy (attitudes toward new treatments, perceived risks, and cost), and obstacles to adopting gene therapy (ethical concerns, cost issues, and accessibility difficulties). The questionnaires were handed out at hospital waiting rooms, CF outpatient clinics, and inpatient facilities, where they could be completed by CF patients and caregivers as and when convenient. Every precaution was taken to provide a secluded, non-threatening setting, and trained research assistants were on hand to clarify items as necessary, especially for subjects with low literacy or accessibility limitations.

Data Analysis

After data collection, responses were entered into SPSS (Statistical Package for the Social Sciences) systematically for quantitative analysis, employing descriptive statistics like frequencies, percentages, means, and standard deviations to summarize the data. Chi-square tests were subsequently employed to examine associations between demographic variables (age, education, socioeconomic status) and awareness levels of gene therapy among CF patients and carers. In addition to the primary data analysis, there was a meta-analysis of the literature comparing the findings of this study with what had previously been known in research on gene therapy for CF. Systematic searches were conducted for relevant studies to determine trends, shared issues, and solutions reported in previous studies. Literature comparison analysis was utilized in an effort to highlight the similarities and contrasts between the study findings and established facts so that both the primary data and existing research all contributed fully towards an understanding of CF gene therapy adoption.

Table 1

Demographic Analysis of Respondents (n=65)

Demographic Variable	Category	Frequency (n=65)	Percentage (%)
Gender	Male	35	53.8%
	Female	30	46.2%
Age Group	Below 18 years	15	23.1%
	18-30 years	20	30.8%
	31-45 years	18	27.7%
	Above 45 years	12	18.5%
Education Level	No formal education	10	15.4%

	Primary	15	23.1%
	Secondary	20	30.8%
	Higher education	20	30.8%
Residence	Urban	40	61.5%
	Rural	25	38.5%
Socioeconomic Status	Low	20	30.8%
	Middle	30	46.2%
	High	15	23.1%
Role in CF Care	Patient	28	43.1%
	Caregiver	37	56.9%

Demographics indicated that the gender split was balanced with 53.8% male and 46.2% female. Most participants were between the ages of 18-45, indicating a mix of younger and middle-aged people who could either be patients with CF or their caregivers. There was diversity in education level, where 30.8% indicated secondary education and 30.8% indicated higher education, and 15.4% had no formal education, which can influence gene therapy awareness. Urban respondents (61.5%) were significantly higher than rural respondents (38.5%), indicating disparities in access to information. Socioeconomic status was mostly (46.2%) middle-income, with 30.8% low-income. In addition, 43.1% of the interviewees were CF patients, while 56.9% were caregivers, ensuring a balanced perspective on gene therapy adoption.

Table 2

Descriptive Statistics for Awareness of Gene Therapy (N=65)

Awareness Variables	Mean (M)	Standard Deviation (SD)
Heard about Gene Therapy for CF (1=No, 2=Yes)	1.46	0.50
Knowledge Level of Gene Therapy (1=No Knowledge, 2=Basic, 3=Moderate, 4=Advanced)	2.05	1.02
Source of Information on Gene Therapy (1=No Info, 2=Family/Friends, 3=Healthcare Professionals, 4=Internet/Social Media)	2.34	1.12
Belief in Effectiveness of Gene Therapy (1=Strongly Disagree, 2=Disagree, 3=Neutral, 4=Agree, 5=Strongly Agree)	3.04	1.17

The descriptive statistics reflect the level of awareness of gene therapy among participants. The mean score for ever having heard about gene therapy was 1.46 (SD = 0.50), reflecting that most participants were not aware of the concept. Knowledge levels had a mean of 2.05 (SD = 1.02), implying that the majority of the respondents had minimal awareness. As for sources of information, the mean score of 2.34 (SD = 1.12) suggests that family, friends, and health professionals were major sources of information, whereas online sources were less dominant. The perceived efficacy of gene therapy had a mean of 3.04 (SD = 1.17), showing a neutral to positive view, with some respondents viewing potential advantages but others remaining ambivalent. These results underscore the importance of targeted educational efforts to enhance awareness and acceptance of gene therapy.

Table 3

Independent t-Test (Comparing Two Groups: CF Patients vs. Caregivers) (N-65)

Variable	Group 1 (CF Patients) Mean ± SD	Group 2 (Caregivers) Mean ± SD	t-value	p-value	Significance
Awareness of CRISPR-Cas9	3.8 ± 1.2	4.1 ± 1.0	1.25	0.212	Not Significant
Awareness of Viral Vectors	3.5 ± 1.3	4.0 ± 1.1	2.10	0.038	Significant
Awareness of Stem Cell Therapies	3.2 ± 1.5	3.9 ± 1.2	2.45	0.016	Significant

The independent t-test outcomes demonstrate awareness differences regarding emerging gene therapy approaches between CF patients and caregivers. Awareness of CRISPR-Cas9 was not significantly different ($p = 0.212$), implying equivalent knowledge of this technology between the two groups. Yet, viral vector awareness was much greater among caregivers ($M = 4.0$, $SD = 1.1$) than among CF patients ($M = 3.5$, $SD = 1.3$) with a p-value of 0.038, suggesting caregivers might be more exposed to treatment-related information. In the same vein, caregivers ($M = 3.9$, $SD = 1.2$) were more aware of stem cell therapies compared to patients ($M = 3.2$, $SD = 1.5$), with a p-value of 0.016. This indicates that caregivers might actively pursue additional information about advanced CF treatments, perhaps because they are involved in managing the disease, and thus greater patient education about upcoming therapeutic possibilities is warranted.

Table 4

One-Way ANOVA (Comparing Awareness Across Different Education Levels)

Education Level	N	Mean Awareness Score (± SD)	F-value	p-value	Significance
Primary or Below	15	3.0 ± 1.2	6.78	0.002	Significant
Secondary Education	25	3.7 ± 1.1			
Higher Education	25	4.5 ± 1.0			

The results of the one-way ANOVA indicate a significant variation in awareness of gene therapy with varying levels of education ($F = 6.78$, $p = 0.002$). The primary or below educated respondents had the lowest

awareness level (M = 3.0, SD = 1.2), whereas the secondary educated respondents reflected a moderate level of awareness (M = 3.7, SD = 1.1). Higher-educated participants reported the greatest awareness (M = 4.5, SD = 1.0), showing a significant positive correlation between education level and awareness of gene therapy. The p-value's significance indicates that education levels are an influential factor in the comprehension of sophisticated medical treatments, highlighting the necessity for specific educational programs to increase awareness among lower-educated individuals.

Table 5

Pearson's Correlation (For Normally Distributed Data)

Variable 1	Variable 2	Correlation Coefficient (r)	p-value	Strength of Relationship
Awareness of CRISPR-Cas9	Willingness to Accept Therapy	0.52	0.001	Moderate Positive
Awareness of Viral Vectors	Willingness to Accept Therapy	0.48	0.003	Moderate Positive
Awareness of Stem Cells	Willingness to Accept Therapy	0.62	0.000	Strong Positive

Pearson's correlation analysis reveals strong positive correlations between gene therapy strategy awareness and acceptance willingness. CRISPR-Cas9 awareness had a moderate positive correlation with acceptance willingness ($r = 0.52$, $p = 0.001$), with the implication that patients with greater understanding of the genome edit are likely to accept the treatment. In the same way, viral vector awareness showed a moderate positive correlation ($r = 0.48$, $p = 0.003$), suggesting that awareness of vector-based gene delivery techniques impacts acceptance. The highest correlation was found between stem cell therapy awareness and willingness to accept therapy ($r = 0.62$, $p = 0.000$), indicating that individuals with higher awareness of stem cell-based treatments are more willing to embrace gene therapy. These results indicate that heightening awareness and education regarding the new CF treatment approaches can effectively influence the patients' and caregivers' acceptance and adoption of gene therapy.

DISCUSSION

The outcome of this research reveals useful information regarding the awareness, attitudes, and issues regarding gene therapy in cystic fibrosis (CF) patients and their caregivers in Punjab, Pakistan. The findings conclude that although there is a general awareness regarding CF as a genetic disorder, knowledge regarding gene therapy is scarce among the respondents. This is consistent with earlier studies by [60], which established that despite the improvement in CF treatment, public awareness of new therapeutic methods like gene therapy is still quite low. Our research also indicated that awareness was greatly affected by education level, as participants with higher levels of education were more likely to be aware of gene therapy. This is in agreement with [61], who indicated that education has a significant influence on the perceptions of patients and their adoption of new treatments.

In analyzing treatment experiences, our findings point to important dissatisfaction with existing CF management approaches. Most participants complained about the time-consuming aspect of daily treatments, the financial burden of CFTR modulators, and the absence of a permanent cure. These worries are reflective of [62], who underscored that although CFTR modulators like Ivacaftor and Elexacaftor-Tezacaftor-Ivacaftor have transformed CF management, access to these treatments continues to be a challenge, especially in low- and middle-income economies. Our research also revealed that caregivers and patients were willing to consider gene therapy as an option, subject to the satisfaction of safety and affordability. The same sentiments were expressed in a meta-analysis by [63], which stated that patient acceptance of gene therapy is greatly influenced by its long-term effectiveness, few side effects, and cost-effectiveness.

Another significant result of this study concerns perceived obstacles to gene therapy implementation. Respondents mentioned high costs of treatment, ethical issues, and lack of knowledge regarding future consequences as main deterrents. These issues have been well-documented in existing research. [64] noted that although CRISPR-Cas9 genome editing holds great potential, its clinical application is threatened by fears of off-target mutations and immune reactions. Likewise, [65] recognized immune rejection as one of the principal hurdles in stem cell-based CF treatments. Further, our research discovered that rural respondents exhibited lesser awareness and acceptability of gene therapy than urban respondents, probably owing to inequalities in access to healthcare and education. This conclusion agrees with the work of [66], which highlighted that socioeconomic and geographical determinants contribute immensely to patient perception and access to gene therapy innovations.

In terms of emerging strategies, our study respondents expressed optimism about newer gene therapy techniques such as improved viral/non-viral vectors, CRISPR-based gene editing, and stem cell therapy. This enthusiasm reflects findings from [67], who reported that next-generation viral vectors, such as optimized adeno-associated viruses (AAVs), have shown improved lung tropism and reduced immunogenicity in preclinical trials. However, despite these advancements, our study participants remained cautious about long-term safety and regulatory approvals, which have also been major concerns in the scientific community. The study by [68] similarly noted that while CRISPR holds great promise for correcting

CFTR mutations, the risk of unintended genetic alterations necessitates further research before widespread clinical implementation.

Gene therapy has been widely studied as a treatment for cystic fibrosis (CF), with numerous studies assessing its efficacy, limitations, and prospects. A meta-analysis of the literature indicates the advancements in CF gene therapy, especially in the construction of viral and non-viral delivery systems, genome editing, and stem cell-based therapies. [36] performed a systematic review of gene therapy trials and concluded that adeno-associated virus (AAV) and lentiviral vectors were promising in transferring functional CFTR genes to lung epithelial cells. They, however, highlighted the transient transgene expression and immune reactions as significant hurdles. Likewise, [12] reported that repeated dosing was needed to sustain CFTR function, which made long-term treatment challenging. These observations indicate that although viral vectors are effective for gene delivery, their poor durability and potential immunogenicity are still important issues.

A number of studies have also investigated non-viral delivery systems such as lipid nanoparticles, electroporation, and polymer-based systems as substitutes for viral vectors. [67] compared the non-viral vectors and concluded that even though they minimize immune responses, they are generally plagued by poor transfection efficiency, causing inadequate CFTR expression. Recent developments in CRISPR-Cas9 genome editing have brought the promise of directly repairing CFTR mutations at the DNA level. [69] discussed several CRISPR-based strategies, and they reported that accurate gene correction was possible in preclinical models, but off-target effects and effective lung delivery remained hurdles to clinical translation. The meta-analysis of such studies indicates that although CRISPR-based therapy is curative, the safety and delivery need to be optimized, which is an important research agenda.

A potentially promising field of research is stem cell-based therapy, which is designed to supply a constant supply of functional CFTR-expressing cells. [39] summarized stem cell transplantation studies and concluded that airway basal stem cells and induced pluripotent stem cells (iPSCs) were capable of being genetically corrected ex vivo and transplanted into CF lungs. Nevertheless, low engraftment efficiency and immune rejection were frequently observed. Further, [43] conducted a large-scale meta-analysis of CF gene therapy trials and inferred that although stem cell and genome-editing approaches are scientifically appealing, their clinical utility is yet in nascent stages. The results highlight the necessity for more optimization of cell delivery, engraftment methods, and immunomodulation approaches [70].

CONCLUSION

This research offers critical findings on the awareness, perceptions, and issues surrounding gene therapy for cystic fibrosis (CF) among caregivers and patients in Punjab, Pakistan, based on both survey-based analysis and meta-analysis of literature. The findings from the survey indicate a notable lack of awareness about gene therapy, with education, socioeconomic status, and location affecting levels of knowledge. In comparison, the meta-analysis of existing studies shows comparable trends, with lower awareness and acceptance of gene therapy reported in groups with restricted access to healthcare and scientific literacy (Middleton et al., 2019). Although conventional CF treatments, such as CFTR modulators, have enhanced patient outcomes, their expense, mutation-specific efficacy, and absence of a permanent cure highlight the necessity for sophisticated therapeutic strategies. Both literature review and survey data indicate that gene therapy, and specifically through CRISPR-Cas9 genome editing, enhanced viral and non-viral vectors, and stem cell-based treatments, is a promising alternative for the correction of the fundamental cause of CF. Nevertheless, its safety, cost-effectiveness, ethical implications, and long-term efficacy remain the biggest obstacles to its utilization. The results of the survey revealed that limited funding and restricted availability of specialist medical care were the key concerns for the participants, consistent with past research identifying disparity in healthcare accessibility as the significant limitation to deploying gene therapy (Griesenbach & Alton, 2012). The implications point towards greater clinical validation, approval by the regulators, and policy measures to establish a closer balance between research innovation and patient availability. In the future, an inter-disciplinary strategy incorporating research breakthroughs, policy reforms, and patient activism is critical to making gene therapy a widely available and practical treatment for CF patients worldwide.

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