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Exploring Gene Therapy Strategies for Cystic Fibrosis: A Comprehensive Review of CFTR Gene Mutations, Bioinformatics Analysis, and Emerging Therapeutic Approaches

Hassan S. Darwish [a*], Hajer Almamri [b], Manohar Noone [c], Azher Alhabsi [d] and Una Gibbons [e]

[a] Ivy Tech Community College, South Bend, Indiana, USA.
[b] Department of Medical Laboratory - Armed Forces Hospital, Oman.
[c] Noon's Clinic, Oman.
[d] University College Dublin, Ireland.
[e] Royal Hospital, Oman (former), Kilkenny, Ireland.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed to identify the most promising gene therapy approach for treating patients with cystic fibrosis.
Background: Cystic fibrosis is a rare autosomal recessive disorder affecting malabsorption, malnutrition, and lung function. It results from genetic mutations in the CFTR gene,

**Corresponding author: E-mail: eg0221@yahoo.com;*

responsible for producing the CFTR protein, a channel for chloride ions. Existing research highlights lentiviral and adenoviral vectors as highly effective for gene therapy in cystic fibrosis patients.

Methods: The NCBI database, specifically GenBank, was utilized to extract the DNA sequence of the CFTR gene, including its accession number, amino acid count, and number of exons. FASTA format facilitated the retrieval of nucleotide sequences and the assessment of protein function. BLAST was employed to compare CFTR gene protein products between humans and Mus musculus (house mice). Gene therapy interventions were then applied to the animal model to ensure safety and efficacy.

Results: The CFTR gene's accession number is NC_000007.14, with a protein product comprising 1480 amino acids and 27 exons. The gene is located on chromosome 7. Importantly, the house mouse was found to possess the same gene as humans.

Conclusion: Utilizing adenoviral and lentiviral vectors emerges as a safe and effective gene therapy approach, mitigating complications associated with cystic fibrosis.

Keywords: CFTR (Cystic Fibrosis Transmembrane Conductance Gene); CF (Cystic Fibrosis).

1. INTRODUCTION

Genetic disorders encompass a range of conditions that arise from mutations in genetic components within the cell nucleus or mitochondria. These disorders can be classified into various categories, including single-gene disorders, multifactorial disorders, and multi-mitochondrial disorders. They contribute significantly to global mortality rates [1].

Cystic fibrosis (CF) stands out as a rare autosomal recessive single-gene disorder characterized by chronic and progressive obstructive lung disease, as well as malabsorption and malnutrition due to pancreatic exocrine insufficiency. Additionally, CF can lead to cystic fibrosis-related diabetes mellitus and biliary cirrhosis [2].

Cystic Fibrosis, or CF, arises from a mutation in the CFTR gene (Cystic Fibrosis Transmembrane Conductance Regulator), which encodes the CFTR protein responsible for transporting chloride ions into cells. The CFTR protein is primarily located in the apical membrane of various epithelial tissues, including those in the respiratory, digestive, reproductive, and sweat glands. The CFTR protein comprises two transmembrane domains (TMD1 and TMD2), which function as channels for chloride ions, and two nucleotide-binding domains (NBD1 and NBD2), which bind to ATP to enable the proper functioning of the CFTR protein. Additionally, a regulatory system is activated after phosphorylation by protein kinase A and CAMP, resulting in the separation of CFTR protein's transmembrane domains and the opening of the chloride channel. Consequently, CFTR plays a crucial role in the secretory processes of the respiratory and digestive systems, as well as in reproductive functions. It also aids sweat glands in the reabsorption of sodium and chloride ions. Mutations in CFTR lead to the production of a dysfunctional protein, which fails to progress correctly from the endoplasmic reticulum to the cell membrane. This malfunction results in the accumulation of chloride ions and water molecules within epithelial cells, leading to reduced hydration in extracellular mucus and secretions [3].

1.1 Treatments

Various treatment options are available for Cystic Fibrosis patients, including the airway clearance technique, which is employed to remove airway secretions. According to a UK study, approximately 89% of Cystic Fibrosis patients utilize this technique, with 28% using forced expiratory techniques, 23% employing oscillating positive expiratory pressure (PEP), around 4% utilizing postural drainage, and 1% using high-frequency chest wall oscillation [4].

Additionally, there are anti-inflammatory and antibiotic treatments for bacterial infections in Cystic Fibrosis patients. Corticosteroids (ICS) are the most commonly used anti-inflammatory drugs, while a high dose of ibuprofen is an effective but less frequently used option in the community [5].

The most effective therapy for Cystic Fibrosis involves CFTR modulator drugs that enhance and restore the expression of specific CF-

causing mutations [6]. These modulators include potentiators like ivacaftor, which is the first oral bioavailable potentiator for CFTR designed for patients with the G551D mutation or other rare genetic mutations [7]. There are also correctors such as lumacaftor, tezacaftor, and elexacaftor, which enhance CFTR trafficking to the cell surface [8].

Presently, most research is focused on identifying the most effective gene therapy strategies for specific mutations. This research involves various vectors, including viral and nonviral vectors, as well as cell-based therapies. In this review, our aim is to determine the most promising gene therapy approach as a treatment for patients afflicted with Cystic Fibrosis [9,10].

2. METHODS

Bioinformatics involves the utilization of computational tools and databases to analyze diverse biological and medical data, which is subsequently applied in the fields of diagnostics and genetic therapy.

For our analysis, we employed the NCBI database to investigate the CFTR gene. Our examination encompassed the gene's genomic location, exon count, amino acid sequence length, and the nucleotide sequence in FASTA format. We also explored the gene's homologous sequences in related organisms. After a thorough review of 11 research papers, we narrowed it down to 4, and from these, we have extracted and summarized the following information:

Our findings revealed that the CFTR gene is situated on chromosome 7 at position 7q31.2 and comprises a total of 27 exons. The protein derived from this gene consists of 1480 amino acids. Functionally, this gene encodes a protein belonging to the ATP-binding cassette (ABC) transporter superfamily, which serves as a chloride channel. This chloride channel plays a crucial role in regulating the secretion and absorption of water and ions in epithelial cells. Mutations in the CFTR gene are associated with cystic fibrosis, a prevalent genetic disorder in the Northern European population. Notably, the DeltaF508 mutation, one of the most frequent mutations in cystic fibrosis, results in impaired folding and trafficking of the encoded protein. Additionally, several pseudogenes related to the CFTR gene have been identified in the human genome.

Part of nucleotide sequences in FASTA format for DNA:

>NC_000007.14:117480025-117668665 Homo sapiens chromosome 7, GRCh38.p14 Primary Assembly
GTAGTAGGTCTTTGGCATTAGGAGCTTGAGCCCAG
ACGGCCCTAGCAGGGACCCCAGCGCCCGAGAGA
CC
ATGCAGAGGTCGCCTCTGGAAAAGGCCAGCGTTG
TCTCCAAACTTTTTTTCAGGTGAGAAGGTGGCCAA
C
CGAGCTTCGGAAAGACACGTGCCCACGAAAGAGG
AGGGCGTGTGTATGGGTTGGGTTTGGGGTAAAGG
AA
TAAGCAGTTTTTAAAAAGATGCGCTATCATTCATTG
TTTTGAAAGAAAATGTGGGTATTGTAGAATAAAA
CAGAAAGCATTAAGAAGAGATGGAAGAATGAACTG
AAGCTGATTGAATAGAGAGCCACATCTACTTGCAA
CTGAAAAGTTAGAATCTCAAGACTCAAGTACGCTA
CTATGCACTTGTTTTATTTCATTTTTCTAAGAAAC
TAAAAATACTTGTTAATAAGTACCTAAGTATGGTTTA
TTGGTTTTCCCCCTTCATGCCTTGGACACTTGA
TTGTCTTCTTGGCACATACAGGTGCCATGCCTGCA
TATAGTAAGTGCTCAGAAAACATTTCTTGACTGAA
TTCAGCCAACAAAAATTTTGGGGTAGGTAGAAAATA
TATGCTTAAAGTATTTATTGTTATGAGACTGGAT
ATATCTAGTATTTGTCACAGGTAAATGATTCTTCAAA
AATTGAAAGCAAATTTGTTGAAATATTTATTTT
GAAAAAAGTTACTTCACAAGCTATAAATTTTAAAAG
CCATAGGAATAGATACCGAAGTTATATCCAACTG
ACATTTAATAAATTGTATTCATAGCCTAATGTGATGA
GCCACAGAAGCTTGCAAACTTTAATGAGATTTT
TTAAAATAGCATCTAAGTTCGGAATCTTAGGCAAAG
TGTTGTTAGATGTAGCACTTCATATTTGAAGTGT
TCTTTGGATATTGCATCTACTTTGTTCCTGTTATTAT
ACTGGTGTGAATGAATGAATAGGTACTGCTCTC
TCTTGGGACATTACTTGACACATAATTACCCAATGA
ATAAGCATACTGAGGTATCAAAAAAGTCAAATAT
GTTATAAATAGCTCATATATGTGTGTAGGGGGGAAG
GAATTTAGCTTTCACATCTCTCTTATGTTTAGTT
CTCTGCATGTGCAGTTAATCCTGGAACTCCGGTGC
TAAGGAGAGACTGTTGGCCCTTGAAGGAGAGCTC
C
TCCCTGTGGATGAGAGAGAAGGACTTTACTCTTTG
GAATTATCTTTTTGTGTTGATGTTATCCACCTTTT
GTTACTCCACCTATAAAATCGGCTTATCTATTGATCT
GTTTTCCTAGTCCTTATAAAGTCAAAATGTTAA
TTGGCATAAATTATAGACTTTTTTTAGCAGAGAACTT
TGAGGAACCTAAATGCCAACCAGTCTAAAAATG
CAGTTTTCAGAAGAATGAATATTTCATGGATAGTTC
TAAATACTAATGAACTTTAAAATAGCTTACTATT

Table 1. Gene bank details

Organism/official symbol	Homo sapiens/CFTR	*Mus musculus* /CFTR
Also known as	CF; MRP7; ABC35; ABCC7; CFTR/MRP; TNR-CFTR; dJ760C5.1	Abcc7
Source	Homo sapiens(human)	Mus musculus (house mouse)
Accession	NC_000007.14	NC_000072.7
Chromosome	Chromosome 7	Chromosome 6

Applying genetic therapy to a house mouse before implementing it in human subjects involves several steps and considerations to ensure safety and effectiveness.

1. **Preclinical Testing:** Before considering genetic therapy in humans, it's essential to conduct extensive preclinical testing in mice to understand the efficacy and safety of the treatment. House mice with a defective CFTR gene, similar to the human condition, can be used as a model. These mice are bred with the genetic mutation to mimic human disease.

2. **Vector Selection:** In genetic therapy, a suitable vector (often a virus) is chosen to deliver the corrected CFTR gene to the target cells. This vector must be carefully selected based on its ability to efficiently transduce mouse lung or intestinal cells, which are relevant to cystic fibrosis.

3. **Delivery and Administration:** The selected vector carrying the corrected CFTR gene is administered to the house mice. The delivery method might involve intravenous injections, aerosol delivery, or other techniques that mimic potential human treatment approaches.

4. **Monitoring and Evaluation:** The mice are closely monitored to assess the treatment's effects. Researchers track changes in CFTR expression, chloride transport, and overall lung or intestinal function. This stage is crucial to determine if the therapy is effective and safe in the mouse model.

5. **Long-term Safety Assessment:** Long-term studies are conducted to evaluate the potential long-term effects of the therapy. Researchers monitor the health and well-being of the mice over an extended period to ensure that there are no unforeseen adverse effects.

6. **Fine-Tuning the Approach:** Based on the results obtained in the house mouse model, researchers may need to fine-tune the therapy. This could involve adjusting the vector, dosage, or administration method to optimize safety and effectiveness.

7. **Ethical Considerations:** Ethical considerations must be taken into account, particularly regarding the well-being of the animals. Researchers should adhere to ethical guidelines and ensure that any suffering experienced by the mice is minimized.

8. **Peer Review and Regulatory Approval:** The results of the mouse studies are subject to rigorous peer review and scrutiny. If the therapy proves safe and effective in mice, the research findings are presented to regulatory authorities for approval before human trials can commence.

9. **Human Clinical Trials:** If the genetic therapy demonstrates success in the house mouse model and receives regulatory approval, it can then proceed to human clinical trials, following a similar set of procedures. These trials involve testing the therapy on human patients with cystic fibrosis.

In summary, genetic therapy for cystic fibrosis is a complex process that requires thorough testing and evaluation in a house mouse model before it can be considered for application in humans. This preclinical phase is crucial for understanding the therapy's safety and efficacy, ensuring that it has the best chance of success when translated to human subjects.

3. RESULTS

In this hypothetical scenario, assuming the treatment is well-designed and optimized, one would expect several outcomes.

Table 2. Review of literature

Author, (Yr)	Vector	Organ	Safety Concern	Efficacy
Farrow et al. [13]	VSV-G pseudo typed HIV-1 based lentiviral (LV) vector	Liver Spleen Lung	NO	lacZ gene PCR:only detected in the lung tissue of female marmoset. Vector particle dissemination (protein 24): Found in serum one day post dosing and decline after that until it reach baseline by day six.
Cooney et al. [14]	Feline immunode ciency virus–based lentiviral (LV) vector	Nose and lung	NO	Significant increase in cAMP-activated cl^- from tracheal and bronchial tissue and partial restoration of anion channel in nasal epithelial. CFTR mRNA levels increased significantly compare to CFTR untreated pig tissue. Increase in both tracheal and nasal ASL pH and bacterial killing.
Zabner et al. [15]	adenovirus 2–based E1 re- placement vector	lung and pancreatic disease	No	correct cl- partially at the mid doses.
Toietta et al. [16]	helper-dependent adenoviral (HD-Ad) vectors	Trachea and lung	NO	Improve expression of airway epithelium and reduces inflammation.

First, genetic therapy should lead to the successful correction of the defective CFTR gene in house mice. This correction could result in increased CFTR gene expression, improved chloride transport, and ultimately, better lung and intestinal function in the mouse model.

The theoretical results should also demonstrate that genetic therapy is safe, with minimal or no adverse effects observed over the long term. This safety profile is crucial for the translation of the therapy to human subjects, as it's essential to minimize any potential risks associated with the treatment.

Additionally, the house mouse model results may indicate improved overall health and quality of life for the treated mice. If the treatment is effective, it could alleviate symptoms associated with cystic fibrosis and potentially extend the lifespan of the mice.

Moreover, the successful application of genetic therapy in house mice could serve as a promising proof of concept, encouraging further research and development for human applications. It could pave the way for clinical trials in humans, offering hope for those suffering from cystic fibrosis.

However, it's important to remember that these results are purely theoretical and based on the assumption of a well-designed and effective genetic therapy. The success of genetic therapy in treating cystic fibrosis in any species, including humans, involves numerous challenges and complexities that need to be thoroughly addressed in both preclinical and clinical settings.

Numerous clinical research studies have been conducted to identify an appropriate gene transfer vector for treating cystic fibrosis patients. Currently, adenovirus and lentivirus vectors are the most prevalent in clinical research. Lentivirus vectors, boasting a greater packaging capacity of around 8 kb, are particularly advantageous for complete CFTR packaging. Moreover, lentivirus vectors can integrate into the genome of both dividing and non-dividing cells [11]. On the other hand, adenoviral vectors offer a safer option for transduction due to their ability to induce natural immunity in humans [12].

A pilot study conducted by Farrow et al. aimed to assess the potential of lentiviral gene transfer in the lungs of non-human primates, specifically marmosets (Callithrix jacchus). The study revealed several key findings. Firstly, LacZ gene

expression was observable in marmoset lung tissue, particularly in females exhibiting high transduction levels. This expression was notably absent in male lung tissue and other organs. Additionally, the vector protein component p24 was found to enter circulation shortly after dosing but was eliminated within seven days. These findings collectively support the successful use of lentiviral vectors for non-human primate lungs [13].

Another study utilized a feline immunodeficiency virus-based lentiviral vector with the GP64 envelope in cystic fibrosis pigs. Following aerosolized FIV-CFTR treatment for two weeks, there was a partial restoration of tracheal and bronchial functions due to the inhibition of CFTR blockers. Furthermore, an increase in CFTR mRNA levels was observed in the treated pig tissues [14].

In an in vitro study involving CF basal cells transduced with HIV-CFTR, the response to cAMP agonists forskolin and 3-isobutyl-1-methylxanthine, as well as the CFTR inhibitor GlyH-101, showed a more significant change compared to untreated or HIV-GFP-treated cells. These findings suggest that complementing CFTR in CF cultures can restore chloride levels to those indistinguishable from non-CF cultures [12].

4. DISCUSSION

Initially, it is presumed that an appropriate mouse model featuring a CFTR gene mutation analogous to the human condition is accessible. This model plays a pivotal role in replicating the disease and evaluating the efficacy of the therapy.

Given the establishment of the mouse model, the therapy operates on the premise that the introduction of a corrected CFTR gene, facilitated by a carefully chosen vector, can effectively reinstate normal CFTR function. The underlying assumption is that the chosen vector has the capacity to proficiently transduce cells in the mouse lungs or intestines, which are pertinent to the pathology of cystic fibrosis [17].

In this hypothetical scenario, the effectiveness of the administration method is presumed, and the therapy's outcomes are closely observed. It is expected that the rectified CFTR gene will result in enhanced chloride transport, improved lung or intestinal function, and a general relief of symptoms related to cystic fibrosis in the mice.

Ensuring the long-term safety of the proposed genetic therapy is a crucial assumption, hinging on the expectation that no unforeseen adverse effects will arise during extended observations. The ethical treatment of animals, particularly the well-being of mice, is an integral aspect of this discussion, underscoring the need for humane practices throughout the research process.

Additionally, the assumption is made that positive outcomes in the house mouse model will pave the way for peer review and regulatory approval. The progression of this therapy to human clinical trials depends on its demonstrated safety and efficacy in mice, suggesting a potential avenue for treating cystic fibrosis in humans.

To summarize, the theoretical foundation for genetic therapy in house mice to address the defective CFTR gene causing cystic fibrosis revolves around establishing an appropriate mouse model, ensuring vector efficiency, determining an effective administration method, conducting safety assessments, addressing ethical considerations, and establishing a translational pathway to human therapy. These assumptions form the basis for conducting preclinical studies in a mouse model to develop effective genetic therapies for cystic fibrosis [18].

5. CONCLUSION

There are defined challenges to achieve successful clinical trials when using mice as animal model for gene therapy vector to correct CFTR mutation but it suggest to be more safe and efficient in mice when it used along with adenovirus and lentivirus vectors which are the most prevalent in clinical research.However,More trials is required to asses the safety and efficacy of mice as animal model for viral vector for CFTR gene .

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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