Rare Diseases: Molecular Mechanisms Underlying Drug Discovery in Cystic Fibrosis

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Aanya Bharadwaj *is a highly motivated high school student with a strong passion for biomedical research, committed to addressing real-world challenges in rare diseases through innovation, scientific inquiry, and translational research.*

1. Introduction:

According to the National Institutes of Health, cystic fibrosis is the most common genetic disorder among Caucasians. Although fewer than 200,000 people in the United States are affected, making it rare, the impact is significant *[1, 2, 3]*. **(Figure 1)** This inherited condition leads to the production of thick, sticky mucus that clogs various organs, especially the lungs and pancreas. Cystic fibrosis results from a mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, specifically the deletion of phenylalanine at the 508th amino acid position *[5, 15]*. **(Figure 2)** This mutation causes the CFTR protein to misfold, prompting the cell to destroy it before it can reach the membrane and function as a chloride ion channel.

2. Molecular Basis of Cystic Fibrosis and CFTR Protein Dysfunction

Misfolded CFTR proteins are identified and disposed of by the cell's quality-control system known as the Endoplasmic Reticulum (ER)–Associated Degradation (ERAD) pathway. In the Endoplasmic Reticulum, helper proteins called chaperones—like Hsp70, Hsp90, and calnexin—try to fold the CFTR protein correctly and prevent any clumping. However, with mutations like ΔF508, the protein becomes too unstable, and these chaperones fail to properly fold CFTR. When this happens, enzymes such as the ubiquitin ligase CHIP attach a ubiquitin "tag" that marks the faulty CFTR protein for removal. This tagged protein is then removed from the ER and delivered to the proteasome, a protein complex where it is broken down into amino acids. This system prevents damaged proteins from reaching the cell surface, but it also destroys nearly all CFTR proteins—including the small portion that might still function. Because of this, less than 1% of the mutant CFTR ever reaches the cell membrane *[25, 27, 28]*. Without functional CFTR, chloride ions cannot exit the cell, leading to a buildup inside and drawing water in through osmosis. **(Figure 3)** The resulting lack of water flow creates thick mucus around the cells, which impairs cilia movement and creates an environment prone to bacterial infection *[6, 10]*. Cystic fibrosis affects more than just the lungs and pancreas; it also impacts the intestines, liver, sweat glands, and reproductive system. In the pancreas, thick mucus blocks the release of digestive enzymes, leading to malnutrition. Patients often require enzyme replacement therapy to help digest food properly. In the liver, mucus can obstruct bile ducts and cause scarring. The sweat glands produce sweat with a higher-than-normal salt concentration, which is why the sweat test remains a standard diagnostic tool for cystic fibrosis. In the reproductive system, many men are infertile due to the absence of certain reproductive tubes. These widespread complications make cystic fibrosis a disease that affects the entire body, not just the lungs *[3, 5, 11]*.

Over the decades, advancements in CF research have significantly improved patient outcomes. The discovery of the CFTR gene in 1989 marked a turning point in understanding the molecular basis of the disease *[13, 15]*. Researchers and institutions such as Francis Collins and the Cystic Fibrosis Foundation played important roles in mapping the genetic causes and developing targeted therapies *[10, 16]*.

3. Pathophysiology of Mucus Accumulation and Multisystem Effects

The discovery of the CFTR gene made it possible to develop drugs that target the root cause of the disease instead of only the symptoms. This included developing ways to fix the mutation itself *[12, 17]*. Ivacaftor, approved in 2012, was the first drug to improve the function of defective CFTR proteins. It worked for patients with certain mutations and manually controlled the opening and closing of the ion channels. Later, drugs combined ivacaftor with other compounds such as lumacaftor and tezacaftor. These drugs helped the misfolded protein reach the cell surface, where it could work as a channel *[12, 17]*. In 2019, the combination therapy called Trikafta was approved. Trikafta works for most patients, including those with the common ΔF508 mutation. These drugs have improved lung function, lowered the number of infections, and helped patients live longer. However, not all patients can use them because some mutations do not respond. The drugs are also expensive, which limits access for many people *[14, 17]*. Trikafta can cost over $300,000 per year in the US and is far beyond the reach of many patients who lack strong insurance coverage. Even in countries with reliable public healthcare systems, the price strain on the nation's budget leads to delays or restricted usage *[26]*.

One of the main challenges in eradicating cystic fibrosis is the buildup of bacterial biofilms in the lungs, in particular the bacteria Pseudomonas aeruginosa *[7, 8, 18, 24]*. Biofilms can act as structural communities that shield themselves from antibiotics and prohibit any immune system actions. In CF patients, the mucus and lack of movement create a perfect environment for these biofilms to form and thrive. Once these biofilms are established, they become increasingly difficult to eradicate and lead to problems like lung infections, inflammation, and a decline in lung function *[7, 19]*. The use of traditional antibiotics and treatments could eventually lead to antibacterial resistance *[8, 19, 24]*.

Biofilms are strong because the bacteria inside them produce a layer that blocks antibiotics and immune system cells. Inside biofilms, bacteria grow slowly, which also makes antibiotics less effective. The bacteria can share resistance genes, which helps the whole group survive treatments. In cystic fibrosis, biofilms are a serious issue because once bacteria like Pseudomonas aeruginosa establish themselves, they stay for life *[7, 18, 19]*. By the teenage years, many patients are chronically infected with Pseudomonas, and treatment can only control the infection, not remove it completely. This is why infections are the leading cause of lung damage in cystic fibrosis *[5, 7]*.

A newly developed treatment includes the use of salicylate therapy. This method targets bacterial communication rather than attempting to kill the bacteria. Sodium salicylate, a common ingredient in certain painkillers, can interfere with quorum sensing in bacteria like P. aeruginosa. **(Figure 4)** Quorum sensing refers to the chemical signaling that bacteria engage in to coordinate gene expression, formation, and antibiotic resistance mechanisms *[20, 23]*. This ability helps bacteria communicate with each other based on population density. By stopping quorum sensing, salicylates aim to prevent bacteria from forming strong biofilm communities and threaten their ability to defend against immune responses *[21, 22]*.

Salicylate therapy does not work the same way as antibiotics. Instead of killing bacteria, it makes them weaker by stopping their communication signals. Without communication, the bacteria cannot form biofilms or coordinate their defenses. This makes them easier to treat with other drugs. Researchers have tested salicylates with silver compounds and antibiotics. The combination works better than antibiotics alone because the biofilm is broken apart first *[21, 22]*. Salicylate therapy is still being studied, but results show that it could become part of future treatment plans. It may not replace antibiotics, but it could reduce the amount of antibiotics needed and slow the development of resistance *[21, 22, 23]*.

Additionally, salicylate therapy has been explored alongside antibiotics or silver-based antimicrobials. After being exposed to salicylate therapy, weakened biofilms can be killed through silver ions and antibiotics. This provides a novel solution to CF cases that don't rely solely on antibiotics, but a multi-step process centered on salicylate therapy *[21, 22]*.

Finally, salicylate therapy provides a host-friendly, minimally invasive strategy to disrupt

bacterial biofilms in vital organs like the lungs. **(Figure 4)** By targeting the interaction and communication of the bacteria rather than their probable death, it offers support to existing treatments and improves the results *[22, 23]*.

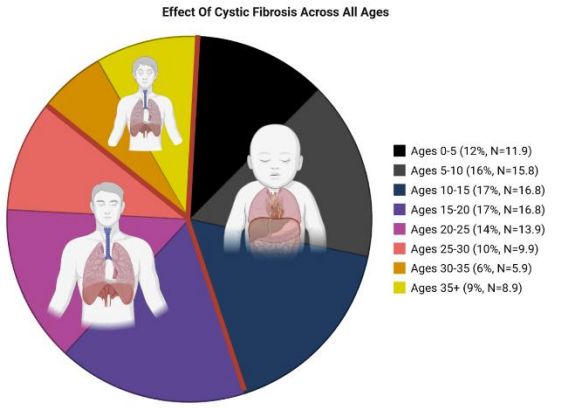

Figure 1: The pie chart above illustrates how cystic fibrosis cases are distributed across different age groups, highlighting how the condition affects individuals throughout their lifespan. The largest proportions are in the 10-15 and 15-20 age groups, followed closely by the 5-10 age group. Overall, the prevalence declines in the older age and is more common at a young age.

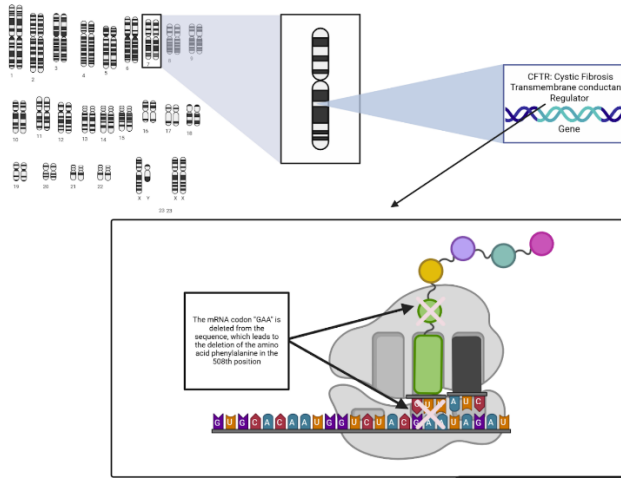

Figure 2: This diagram illustrates the genetic basis and origin of the cystic fibrosis mutation. It focuses on the mutation in the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene located on chromosome 7. The CFTR gene encodes a protein essential for transporting chloride ions across cell membranes and maintaining stable osmosis levels. In individuals with cystic fibrosis, a common mutation—specifically the deletion of the mRNA codon "GAA"—leads to the loss of the amino acid phenylalanine at the 508th position in the amino acid sequence. This defect disrupts proper protein folding and function, resulting in a mutated protein, impairs chloride transport, and leads to the buildup of thick mucus in various organs, especially the lungs and pancreas. The illustration connects chromosomal location to molecular translation, highlighting the role of genetic mutations in the manifestation of cystic fibrosis in patients.

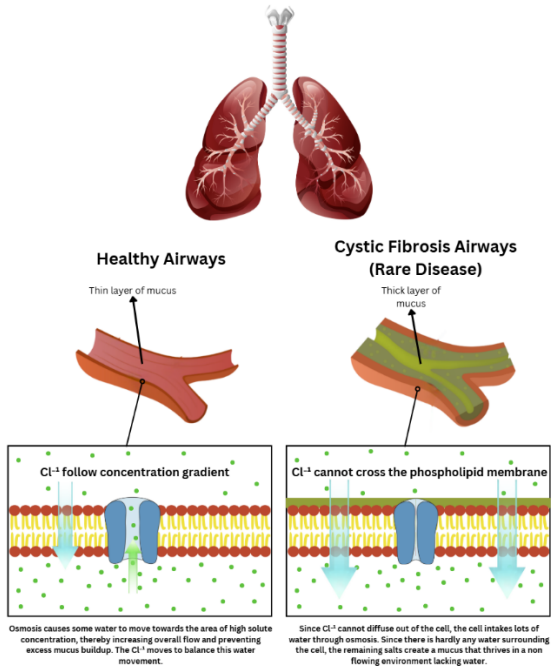

Figure 3: Cystic fibrosis (CF) is a hereditary disease that mainly affects the lungs and digestive system. It is caused by a mutation in the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene. This gene is responsible for producing a protein that controls the movement of chloride ions across the cell membranes in the lungs and other organs. In healthy lungs, these chloride ions help attract water to the surface of the airways through osmosis. This process keeps the mucus in the lungs thin and slippery, which is important for trapping and clearing out dust, bacteria, and other particles. When chloride ions flow out of the cells properly, water follows, and the lungs remain clean and functional.

However, in individuals with cystic fibrosis, the CFTR protein is either missing or does not function properly due to genetic mutations. As a result, chloride ions are unable to

exit the cells. This leads to an imbalance where water is drawn into the cells rather than remaining on the surface of the airways. Without enough water outside the cells, the mucus becomes thick and sticky. This thick mucus clogs the airways, making it hard to breathe and providing an ideal environment for bacteria to grow. Repeated lung infections and chronic inflammation are common, leading to progressive lung damage over time.

4. Advances in Targeted Therapies: CFTR Modulators and Precision Medicine

Researchers are exploring many ways to treat CF, including medications that aim to repair the faulty CFTR protein or help the body produce a better-functioning version of it. One major advancement is the development of CFTR modulators—drugs that improve the function of the defective protein. Additionally, gene therapy holds promise for correcting the genetic defect at its source. Understanding the basic cellular mechanism of CF not only helps in developing treatments but also inspires young researchers to explore how small changes in genes can lead to major health challenges—and how science can work to solve them.

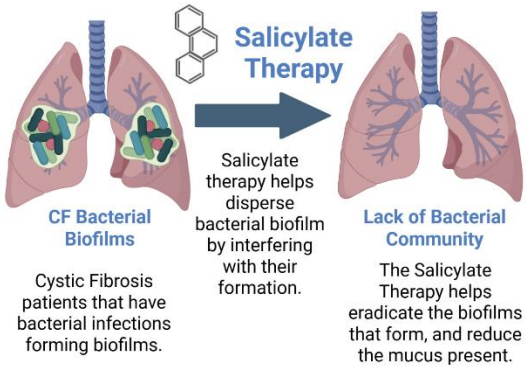

Figure 4: The image showcases the benefits of salicylate therapy in treating cystic fibrosis-associated biofilms. The left lungs are affected by CF and show a growing bacterial biofilm inhibiting lung function. On the right, there is an image of a lung that has gone through salicylate therapy. Salicylate therapy focuses on disrupting bacterial biofilm communication rather than eradicating the bacteria themselves. As a result of the antibacterial treatment after disrupting communication, the lung shows reduced mucus load and less biofilm presence. Overall, salicylate therapy offers a promising solution to disrupting biofilms in cystic fibrosis patients.

5. Conclusion

In conclusion, cystic fibrosis is a complex genetic disorder that affects multiple systems and remains a major challenge despite countless research and advancements. For example, the discovery of the CFTR gene and development of modulators such as Trikafta has transformed care by identifying the root cause and targeting it. However, challenges like infections by bacterial biofilms continue to slow this progress down. Novel approaches like salicylate therapy offer promising solutions to weaken bacterial communication and defenses, enhancing existing treatments. Continued research on this therapy combines genetic, molecular, and microbiological strategies that help overcome these obstacles in cystic fibrosis, allowing this disease to be effectively controlled.

References:

1. Mall, M. A., Burgel, P.-R., Castellani, C., Davies, J. C., Salathe, M., & Taylor-Cousar, J. L. (2024). *Cystic fibrosis*. **Nature Reviews Disease Primers, 10**(1). https://doi.org/10.1038/s41572-024-00538-6

2. Diab Cáceres, L., & Zamarrón de Lucas, E. (2023). *Fibrosis quística: epidemiología, clínica, diagnóstico y tratamiento*. **Medicina Clínica, 161**(9). https://doi.org/10.1016/j.medcli.2023.06.006

3. Ong, T., & Ramsey, B. W. (2023). *Cystic fibrosis: A review*. **JAMA, 329**(21), 1859–1871. https://doi.org/10.1001/jama.2023.8120

4. Myer, H., Chupita, S., & Jnah, A. (2023). *Cystic Fibrosis: Back to the Basics*. **Neonatal Network, 42**(1), 23–30. https://doi.org/10.1891/nn-2022-0007

5. Shteinberg, M., Haq, I. J., Polineni, D., & Davies, J. C. (2021). *Cystic fibrosis*. **The Lancet, 397**(10290), 2195–2211. https://doi.org/10.1016/s0140-6736(20)32542-3

6. Dickinson, K. M., & Collaco, J. M. (2021). *Cystic Fibrosis*. **Pediatrics in Review, 42**(2), 55–67. https://doi.org/10.1542/pir.2019-0212

7. Martin, I., Waters, V., & Grasemann, H. (2021). *Approaches to Targeting Bacterial Biofilms in Cystic Fibrosis Airways*. **International Journal of Molecular Sciences, 22**(4), 2155. https://doi.org/10.3390/ijms22042155

8. Smith, S., Waters, V., Jahnke, N., & Ratjen, F. (2020). *Standard versus biofilm antimicrobial susceptibility testing to guide antibiotic therapy in cystic fibrosis*. **Cochrane Database of Systematic Reviews**. https://doi.org/10.1002/14651858.cd009528.pub5

9. Rafeeq, M. M., & Murad, H. A. S. (2017). *Cystic fibrosis: current therapeutic targets and future approaches*. **Journal of Translational Medicine, 15**(1). https://doi.org/10.1186/s12967-017-1193-9

10. Ratjen, F., Bell, S. C., Rowe, S. M., Goss, C. H., Quittner, A. L., & Bush, A. (2015). *Cystic fibrosis*. **Nature Reviews Disease Primers, 1**(1), 15010. https://doi.org/10.1038/nrdp.2015.10

11. Elborn, J. S. (2016). *Cystic fibrosis*. **The Lancet, 388**(10059), 2519–2531. https://doi.org/10.1016/s0140-6736(16)00576-6

12. Lopes-Pacheco, M. (2016). *CFTR Modulators: Shedding Light on Precision Medicine for Cystic Fibrosis*. **Frontiers in Pharmacology, 7**, 275. https://doi.org/10.3389/fphar.2016.00275

13. Rowe, S. M., Miller, S., & Sorscher, E. J. (2005). *Cystic Fibrosis*. **New England Journal of Medicine, 352**(19), 1992–2001. https://doi.org/10.1056/nejmra043184

14. Bierlaagh, M. C., Muilwijk, D., Beekman, J. M., & van der Ent, C. K. (2021). *A new era for people with cystic fibrosis*. **European Journal of Pediatrics, 180**(9). https://doi.org/10.1007/s00431-021-04168-y

15. Sosnay, P. R., Siklosi, K. R., Van Goor, F., Kaniecki, K., Yu, H., Sharma, N., Ramalho, A. S., Amaral, M. D., … & Penland, C. M. (2013). *Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene*. **Nature Genetics, 45**(10), 1160–1167. https://doi.org/10.1038/ng.2745

16. Goss, C. H., & Ratjen, F. (2013). *Update in Cystic Fibrosis 2012*. **American Journal of Respiratory and Critical Care Medicine, 187**(9), 915–919. https://doi.org/10.1164/rccm.201301-0184up

17. Quon, B. S., & Rowe, S. M. (2016). *New and emerging targeted therapies for cystic fibrosis*. **BMJ, 352**, i859. https://doi.org/10.1136/bmj.i859

18. Chadha, J., Harjai, K., & Chhibber, S. (2021). *Repurposing phytochemicals as anti-virulent agents to attenuate quorum sensing-regulated virulence factors and biofilm formation in Pseudomonas aeruginosa*. **Microbial Biotechnology**. https://doi.org/10.1111/1751-7915.13981

19. De Bleeckere, A., Van den Bossche, S., De Sutter, P.-J., Beirens, T., Crabbé, A., & Coenye, T. (2023). *High throughput determination of the biofilm prevention concentration for Pseudomonas aeruginosa biofilms using a synthetic cystic fibrosis sputum medium*. **Biofilm, 5**, 100106. https://doi.org/10.1016/j.bioflm.2023.100106

20. Hentzer, M., Eberl, L., Nielsen, J., & Givskov, M. (2003). *Quorum Sensing*. **BioDrugs, 17**(4), 241–250. https://doi.org/10.2165/00063030-200317040-00003

21. Gerner, E., Almqvist, S., Thomsen, P., Werthén, M., & Trobos, M. (2021). *Sodium Salicylate Influences the Pseudomonas aeruginosa Biofilm Structure and Susceptibility Towards Silver*. **International Journal of Molecular Sciences, 22**(3), 1060. https://doi.org/10.3390/ijms22031060

22. Gerner, E., Giraldo-Osorno, P. M., Johansson, A., Firdaus, R., Ben Amara, H., Werthén, M., Palmquist, A., Thomsen, P., Omar, O., Almqvist, S., & Trobos, M. (2023). *Targeting Pseudomonas aeruginosa quorum sensing with sodium salicylate modulates immune responses in vitro and in vivo*. **Frontiers in Cellular and Infection Microbiology, 13**. https://doi.org/10.3389/fcimb.2023.1183959

23. Mitra, A. (2024). *Combatting biofilm-mediated infections in clinical settings by targeting quorum sensing*. **The Cell Surface, 12**, 100133. https://doi.org/10.1016/j.tcsw.2024.100133

24. Smith, S., Waters, V., Jahnke, N., & Ratjen, F. (2020). *Standard versus biofilm antimicrobial susceptibility testing to guide antibiotic therapy in cystic fibrosis*. **Cochrane Database of Systematic Reviews**. https://doi.org/10.1002/14651858.cd009528.pub5

25. Krshnan, L., van de Weijer, M. L., & Carvalho, P. (2022). Endoplasmic Reticulum-Associated Protein Degradation. ***Cold Spring Harbor perspectives in biology***, *14*(12), a041247. https://doi.org/10.1101/cshperspect.a041247

26. Guo, J., Hennessy, G., Young, B., & Hill, A. (2025). Strategies used to access CFTR modulators in countries without reimbursement agreements. *Journal of cystic fibrosis : official journal of the* ***European Cystic Fibrosis Society***, *24*(2), 290–294. https://doi.org/10.1016/j.jcf.2025.02.010

27. Valastyan, J. S., & Lindquist, S. (2014). Mechanisms of protein-folding diseases at a glance. ***Disease models & mechanisms***, *7*(1), 9–14. https://doi.org/10.1242/dmm.013474

28. Ganesan, K., Rajaram, K., Rajagopal, T., Fathima, A., Afreena, S. T., Shiyamala Gowri, G., Karthick, P., & Appavu, R. (2025). Tissue Processing for Microscopic Analysis with Applications in Oral Histology, J. Am. Sci. Soc @STEM 1(1); 0098-0103

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