Closer to a Cure

Daniel Erenstein

Writer's Comment: In the fall of 2017, I enrolled in UWP 104E (Writing in Science), taught by Dr. Brenda Rinard. The culmination of a quarter-long research process came in the form of an assignment that asked us to write a popular science article intended for a lay audience. In the early stages of my research, I bounced from topic to topic, struggling to navigate the expansive sphere of scientific literature. With Dr. Rinard's guidance, I settled on an investigation of the challenges in treating cystic fibrosis and the solutions as suggested by recent research. Along the way I learned that science has a unique informative power—but only if translated accurately into accessible language. For all their noteworthy accolades and accomplishments, scientists often struggle to effectively communicate their research to others, including professional peers in their field and the public at large. In today's world, successful science communication is as necessary as ever. As chronicled in this article, the search for absolute answers to difficult questions drives promising innovations. This piece would not have been possible without the inspiring stories of Mary Frey, Nina Wine, and the entire cystic fibrosis community. This story is ultimately theirs.

Instructor's Comment: Daniel's writing on this assignment—explain an area of scientific research to a non-scientist audience—is intensely human. By weaving the story of one family into his discussion of current research on the treatment of cystic fibrosis, Daniel's work shows us that science, and the written communication of it, is deeply personal and relevant to a wide audience. His work illustrates that

without the human element in science, its benefits might go unnoticed by the people who need it most. Daniel's deep commitment to writing, revision, and his attention to small details—from his use of visuals to his careful citations—also make this piece stand out.

—Brenda Rinard, University Writing Program

For more than three years running, Mary and Peter Frey conclude each day by waving goodbye and directing the lens of their video camera towards Mary's dog, Oliver, or Ollie for short. Nothing about the initial appearance of this tight-knit crew indicates that they are anything but a typical family, as viewers on YouTube watch them quarrel over seemingly trivial topics of discussion in their daily video blogs, or vlogs. However, watch further and you are drawn into a beautifully touching story of love, compassion, and optimism in the face of great struggle. It becomes quickly apparent while delving into their video archive that Mary has cystic fibrosis, a genetically-inherited, life-threatening condition that, due to a buildup of mucus, leaves the respiratory and digestive system more prone to chronic bacterial infections. The videos follow Mary, Peter, and Ollie as they navigate a cocktail of pharmaceutical treatments and hospital visits, documenting the evolution of Mary's disease and inviting us to join them on their adventurous journey. Their vlog The Frey Life and their YouTube channel has generated over 40 million views since 2013 with a following of over 200,000 subscribers (1).

Mary is just one of over 70,000 people worldwide with cystic fibrosis, according to the Cystic Fibrosis Foundation Patient Registry (2). In the United States alone, there are over 30,000 cystic fibrosis patients (3), and each cystic fibrosis story is uniquely personal. Numerous scientists have dedicated their lives to studying and researching cystic fibrosis, its symptoms, and the mechanisms that trigger it. But are we any closer to discovering an ever-so-elusive cure for the disease?

Discovering the Cystic Fibrosis Gene

There is no denying the progress made in researching cystic fibrosis over the past few decades. In the summer of 1989, Francis Collins, the former Director of the National Human Genome Research

Institute (NHGRI) as well as the former Project Head of the Human Genome Project, discovered the gene responsible for cystic fibrosis in collaboration with Lap-Chee Tsui, Geneticist-in-Chief at the Hospital for Sick Children in Toronto, and a supporting team of researchers at Yale University. Earlier research had narrowed the location of the cystic fibrosis gene to chromosome 7, the seventh pair of informational, genetic units in a series of 23 such pairs found in humans (3, 4). This is where the Yale University team precisely located the mutated cystic fibrosis gene in 1989 (3, 5, 6, 7).

With the help of biochemist Jack Riordan, the team determined that the gene, now known as the cystic fibrosis transmembrane conductance regulator (CFTR) gene, triggered the expression of faulty chloride ion channels, or tunnels in the membrane boundary of cells that allow for the passage of only select molecules (3, 6). These channels, when fully functional, fulfill the same purpose as a traffic light, allowing chloride "cars" to pass through the intersection when traffic in a certain direction is highly congested. When the channel malfunctions due to a mutation in the CFTR gene, the traffic light is stuck on red, blocking the transit of chloride "cars" across the intersection. An accumulation of chloride ions inside the cell draws water from the outside in an effort to balance the disparity in ion concentrations (3). As a result, mucus on the exterior of the cell is deprived of water, becoming thick and causing congestion in the lungs of cystic fibrosis patients (3, 8, 9).

The final discovery by Collins and Tsui arrived with the identification of the most common CFTR mutation, coined DF_{508} by the team from Yale University (10). DF_{508} is so-named for the normal position of the amino acid—the molecular building blocks of proteins—phenylalanine, which in the mutated form of the protein is absent due to a DNA base pair deletion (11, 12). Imagine the entirely operational version of your CFTR gene as an essay with 1,480 words. If you accidentally delete one word from your essay, the grammar of a particular sentence might be affected, but the essay's overall purpose is not lost. To suggest that one word makes or breaks your essay would be absurd. Incredibly, however, the deletion of one amino acid from the protein encoded by the CFTR gene completely alters the functionality of the ion channel (3).

Collins, now the Director of the National Institutes of Health, and Tsui, Emeritus Professor at the University of Toronto, contributed immensely to the field of cystic fibrosis with their foundational findings.

Thanks to their Yale University team, research on cystic fibrosis surged ahead in the 1990s and 2000s. That said, despite a promising outlook after the discovery of the cystic fibrosis gene and the corresponding DF_{508} mutation, subsequent research left several questions unanswered about the relationship between genotype, our genetic code, and phenotype, the observable physiological characteristics of disease.

Understanding Genotype and Phenotype Relationships

With the knowledge that cystic fibrosis can be explained by mutations in the CFTR gene, researchers today reason that a treatment for the disease is visible on the horizon. On the surface, it certainly seems possible that we will develop a cure for cystic fibrosis in the very near future. However, there exists a contingent of scientists who aren't entirely convinced that the relationship between CFTR mutations and the development of cystic fibrosis is so simple.

A 2015 study conducted by a team of researchers led by Andrew Fraser of the Fraser Lab at the Donnelly Centre for Cellular and Biomolecular Research at the University of Toronto confirmed these fears. To examine the relationship between genotype and phenotype, the researchers experimented with two different geographic isolates of *C. elegans*, a species of nematode (13, 14). A geographic isolate is a group of organisms from the same species, separated from other individuals of the species by a geographic barrier, like a larger body of water or a treacherous mountain range. In each trial, approximately fifteen larvae of each isolate—a Bristol, England isolate and a Hawaiian isolate—were exposed for four days to bacteria that expressed types of double-stranded RNA (dsRNA). Each colony of bacteria was programmed with dsRNA that attacked a certain gene in the roundworm DNA; in other words, exposure to these bacteria caused roundworms to incorporate elements of the bacterial code into their own DNA and to then express the encoded physical characteristics. The researchers used a worm sorter, a device that categorizes the visual properties of worms, to compare the phenotypes of control worms fed normal bacteria to the phenotypes of those worms fed gene-targeting bacteria. After comparing the phenotypes of the Bristol isolate with the Hawaiian isolate, they found that 9 percent of the experimentally modified genes resulted in more intense phenotypic

effects in the Hawaiian isolate and 42 percent of the experimentally modified genes resulted in more intense phenotypic effects in the Bristol isolate (14). If the two isolates of roundworms from the same species were exposed to the same bacterial culture, it is expected that they would express similar levels of each characteristic, not the varied levels seen in the results of the experiment. There is something else besides genotype at play here, perhaps unknown environmental variables or genetic modifiers (15, 16).

What does an assortment of roundworms from England and Hawaii have in common with cystic fibrosis in humans? One might reasonably question the applications of this research to humans, but the universal nature of the genetic code—meaning that it is shared by bacteria, plants, animals, and everything in between—allows us to quite easily apply these findings to human pathology. This is not to say that mutations in the CFTR gene are completely unrelated to the disease. Years of research conclusively confirm that the CFTR genotype and cystic fibrosis phenotype are closely related to each other—and that genotype is often a very useful tool in predicting the development and progression of disease (17, 18). However, an effective understanding of the relationship between genotype and phenotype requires nuance in our scientific approach. In a *ScienceDaily* press release about the Fraser Lab's recent findings, Jovana Drinjakovic, staff writer for the Donnelly Centre, best explains the importance of this understanding: "[W]hile some patients are diagnosed as newborns, others do not show any signs of the disease until adulthood. Predicting disease severity is critical because often the uncertainty can be almost as frightening as the diagnosis" (13).

How, then, can it be that individuals with an identical disease-causing mutation in their genetic sequences experience symptoms of the disease with varying levels of intensity? And why do individuals with the same CFTR mutation sometimes react differently to the same clinical treatment? The solutions to these questions are the key to unlocking a comprehensive treatment and cure for cystic fibrosis. If scientists can understand why the same mutation in the genes of two separate cystic fibrosis patients might result in unexpectedly different responses from the human body, effective medicines and pharmaceutical treatments can be tailored to the individual and their genetic background. This method of attack, often called personalized medicine or precision medicine, holds exciting promise for future developments in the field of cystic fibrosis research.

Using Personalized Medicine to Affect Change

Fortunately, a recent study of the effects of certain drugs on the secretion of sweat from the glands of cystic fibrosis patients is pushing the field of cystic fibrosis forward. Enter Jeffrey Wine, the Benjamin Scott Crocker Professor of Human Biology at Stanford University. Wine's extensive research on the disease in the Cystic Fibrosis Research Laboratory (CFRL) at Stanford is an endeavor of both scientific and personal significance, spurred by an experience with the disease that strikes close to home.

Over forty years ago, Wine arrived at Stanford University as an assistant professor of psychology with a vastly different research focus: neurons, the cells that compose our brain tissue, and the ways in which a system of neurons transmit signals between each other. In 1981, doctors diagnosed his infant daughter Nina with cystic fibrosis, changing the course of Jeffrey's career entirely. Wine shifted gears, examining and analyzing the concurrent, parallel research of his peers, including Francis Collins and Lap-Chee Tsui, to learn more about Nina's disease (19, 20). He is now a regular contributor to the field of research on the disease.

Recently published research by Wine and his Stanford laboratory team has revealed that ivacaftor, previously known during development as VX-770, is effective in treating cystic fibrosis (9). VX-770 is known as a "doorman drug," referring to the drug's function in unlocking the door of the blocked CFTR channel (3). Ivacaftor is one of several drugs currently on the market today that targets deficiencies in the CFTR protein and partially restores its original function. Ivacaftor serves as a mechanic, fixing the defect in the broken traffic light mentioned previously and reactivating the CFTR membrane channel protein.

In response to a treatment of ivacaftor, a sample of cystic fibrosis patients produced a certain type of sweat, which the Stanford team calls C-sweat. Because production of this sweat depends upon a functioning CFTR protein, Wine and company conclude that ivacaftor is a useful treatment for CFTR defects. Most available drugs direct their attention towards the symptoms of cystic fibrosis with little long-term success, whereas ivacaftor targets the molecular mechanisms that cause those symptoms (21). Other similar drugs—such as Kalydeco, a different form of VX-770—perform the same function. Kalydeco, however, is currently undergoing development, as Vertex Pharmaceuticals works to combine

VX-770 with VX-809, also known as the "corrector drug" (3, 8). VX-809 is so-named because it corrects the shape of a deformed CFTR protein that is unable to reach the surface of the cell. The "corrector drug" molds the protein into its normal form, treating the protein like playdough to help it claim its rightful spot in the plasma membrane (3).

Looking Ahead to the Future

In tandem, the teamwork of VX-770 and VX-809 have the potential to revolutionize the treatment of cystic fibrosis. Already the effect of such drugs on cystic fibrosis patients has been, for the most part, very positive, providing cause for scientists and the community to be optimistic about the future of cystic fibrosis research. Still, even considering the development of two more drugs (VX-661 and VX-983) in the pharmaceutical pipeline, we have yet to consider treatments for patients with only one copy of the DF_{508} mutation (3). How do we approach the defects caused by other errors in the CFTR gene, such as the G551D and R117H mutations? Will currently existing drugs take up the challenge, or will entirely new treatments need to be envisioned? The search for a comprehensive cure, pharmaceutical or otherwise, marches forward. For Mary Frey, Nina Wine, and countless other cystic fibrosis patients struggling to survive, no search is of greater significance.

More to Explore

C. Castellani, H. Cuppens, M. Macek Jr., J. J. Cassiman, E. Kerem, P. Durie, E. Tullis, B. M. Assael, C. Bombieri, A. Brown, et al. 2008. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros. 7(3):179-196.

Patrick R. Sosnay, Karen S. Raraigh, Ronald L. Gibson. 2016. Molecular genetics of cystic fibrosis transmembrane conductance regulator: genotype and phenotype. Pediatr Clin North Am. 63(4):585-598.

Patrick R. Sosnay, Danieli B. Salinas, Terry B. White, Clement L. Ren, Philip M. Farrell, Karen S. Raraigh, Emmanuelle Girodon, Carlo Castellani. 2017. Applying cystic fibrosis transmembrane conductance regulator genetics and CFTR2 data to facilitate diagnoses. J Pediatr. 181:S27-S32.

References

1. Mary Frey, Peter Frey. Life with Cystic Fibrosis & a Service Dog [Internet]. 2017. Grafton (MA): The Frey Life; [cited 2017 Dec 5]. Available from https://www.youtube.com/user/thefreylife.
2. About Cystic Fibrosis [Internet]. 2017. Bethesda (MD): Cystic Fibrosis Foundation; [cited 2017 Dec 5]. Available from https://www.cff.org/What-is-CF/About-Cystic-Fibrosis/.
3. Bijal P. Trivedi. Doorway to a Cure for Cystic Fibrosis [Internet]. 2013. Waukesha (WI): Discover Magazine; [cited 2017 Dec 5]. Available from http://discovermagazine.com/2013/september/14-doorway-to-a-cure.
4. Brandon J. Wainwright, Peter J. Scrambler, Jorg Schmidtke, Eila A. Watson, Hai-Yang Law, Martin Farrall, Howard J. Cooke, Hans Eiberg, Robert Williamson. 1985. Localization of cystic fibrosis locus to human chromosome 7cen-q22. Nature. 318:384-385.
5. Johanna M. Rommens, Michael C. Iannuzzi, Bat-sheva Kerem, Mitchell L. Drumm, Georg Melmer, Michael Dean, Richard Rozmahel, Jeffrey L. Cole, Dara Kennedy, Noriko Hidaka, et al. 1989. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science. 245(4922):1059-1065.
6. John R. Riordan, Johanna M. Rommens, Bat-sheva Kerem, Noa Alon, Richard Rozmahel, Zbyszko Grzelczak, Julian Zielenski, Si Lok, Natasa Plavsic, Jia-Ling Chou, et al. 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science. 245(4922):1066-1073.
7. Bat-sheva Kerem, Johanna M. Rommens, Janet A. Buchanan, Danuta Markiewicz, Tara K. Cox, Aravinda Chakravarti, Manuel Buchwald, Lap-Chee Tsui. 1989. Identification of the cystic fibrosis gene: genetic analysis. 245(4922):1073-1080.
8. Deborah M. Cholon, Martina Gentzsch. 2017. Recent progress in translational cystic fibrosis research using precision medicine strategies. J Cyst Fibros. Article in Press.
9. Jessica E. Char, Marlene H. Wolfe, Hyung-ju Cho, Il-ho Park, Jin Hyeok Jeong, Eric Frisbee, Colleen Dunn, Zoe Davies, Carlos

Milla, Richard B. Moss, et al. 2014. A little CFTR goes a long way: CFTR-dependent sweat secretion from G551D and R117H-5T cystic fibrosis subjects taking ivacaftor. PLOS One. 9(2):88564.

10. Wanda K. Lemna, Gerald L. Feldman, Bat-sheva Kerem, Susan D. Fernbach, Elaine P. Zevkovich, William E. O'Brien, John R. Riordan, Francis S. Collins, Lap-Chee Tsui, Arthur L. Beaudet. 1990. Mutation analysis for heterozygote detection and the prenatal diagnosis of cystic fibrosis. N Engl J Med. 322(5):291-296.
11. Eitan Kerem, Mary Corey, Bat-sheva Kerem, Johanna Rommens, Danuta Markiewicz, Henry Levison, Lap-Chee Tsui, Peter Durie. 1990. The relation between genotype and phenotype in cystic fibrosis – analysis of the most common mutation (DF_{508}). N Engl J Med. 323(22):1517-1522.
12. Marcus Mall, Silvia M. Kreda, April Mengos, Timothy J. Jensen, Stephanie Hirtz, Hans H. Seydewitz, James Yankaskas, Karl Kunzelmann, John R. Riordan, Richard C. Boucher. 2004. The DF_{508} mutation results in loss of CFTR function and mature protein in native human colon. Gastroenterology. 126(1):32-41.
13. Jovana Drinjakovic. Why bad genes don't always lead to bad diseases [Internet]. 2015. Rockville (MD): ScienceDaily; [cited 2017 Dec 5]. Available from https://www.sciencedaily.com/releases/2015/07/150716135104.htm.
14. Victoria Vu, Adrian J. Verster, Michael Schertzberg, Tungalag Chuluunbaatar, Mark Spensley, Djina Pajkic, G. Traver Hart, Jason Moffat, Andrew G. Fraser. 2015. Natural variation in gene expression modulates the severity of mutant phenotypes. Cell. 162(2):391-402.
15. Carol Durno, Mary Corey, Julian Zielenski, Elizabeth Tullis, Lap-Chee Tsui, Peter Durie. 2002. Genotype and phenotype correlations in patients with cystic fibrosis and pancreatitis. Gastroenterology. 123(6):1857-1864.
16. E. De Wachter, M. Thomas, S. S. Wanyama, S. Seneca, A. Malfroot. 2017. What can the CF registry tell us about rare CFTR-mutations? A Belgian study. Orphanet J Rare Dis. 12(42).
17. Edward F. McKone, Christopher H. Goss, Moira L. Aitken. 2006.

CFTR genotype as a predictor of prognosis in cystic fibrosis. Chest. 130(5):1441-1447.

18. Edward F. McKone, Priscilla Velentgas, Anna J. Swenson, Christopher H. Goss. 2015. Association of sweat chloride concentration at time of diagnosis and CFTR genotype with mortality and cystic fibrosis phenotype. J Cyst Fibros. 14(5):580-586.
19. Marcia Barinaga. Personal tragedy puts passion back into a scientist's quest [Internet]. 1989. New York City (NY): The Scientist; [cited 2017 Dec 5]. Available from https://www.the-scientist.com/?articles.view/articleNo/10341/title/Personal-Tragedy-Puts-Passion-Back-Into-A-Scientist-s-Quest/
20. Linley E. Hall. Love of his daughter and dedication to scientific study opened a new career path for Jeffrey Wine [Internet]. 2002. Stanford (CA): Stanford Report; [cited 2017 Dec 5]. Available from https://news.stanford.edu/news/2002/july10/wine.html
21. Becky Bach. Stanford researchers' new test for cystic fibrosis may lead to more treatments [Internet]. 2014. Stanford (CA): Stanford Report; [cited 2017 Dec 5]. Available from https://news.stanford.edu/news/2014/february/cystic-fibrosis-test-021114.html