Unraveling cystic fibrosis puzzle, taking it personally matters

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In the genetic disorder cystic fibrosis (CF), the most severe symptoms are recurring episodes of lung inflammation and bacterial infection (known as "exacerbations") that happen from one to three times a year and cause ever-increasing amounts of lung damage through the course of a CF patient's life. While it is well understood that CF lung problems are ultimately due to bacterial infections encouraged by a CF patient's abnormally thick mucus, medical science has been unable to define specific causes that trigger the periodic flare-ups.

In a recent article in the *Journal of Clinical Microbiology*, lead author Joshua Stokell, a post-doctoral researcher in biology, and a team from University of North Carolina at Charlotte describe an unusual, single-patient study and an important finding that may point to an immediate cause of CF exacerbations. The study found sudden growth of a specific bacterium, *Burkholderia multivorans*, preceded periods of acute illness. While *Burkholderia* has been known as a common pathogen in the lungs of CF patients, it is far less abundant than another pathogen, Pseudomonas aeruginosa, whose populations did not show significant changes prior to the life-threatening episodes.

Stokell's co-authors on the paper are UNC Charlotte biologist Todd Steck and UNC Charlotte bioinformaticians Anthony Fodor, Malcolm Zapata, Raad Gharaibeh and Timothy Hamp.

Stokell and his colleagues were able to implicate *Burkholderia* thanks to a detailed genomic and metagenomic analysis of a unique, extensive

collection of lung sputum samples taken twice-weekly over a three-year period.

"This study represents the most intensive sampling of a single CF patient to date," the article notes. "Our study was based upon the assumption that a detectable shift in the bacterial community precedes a pulmonary exacerbation. Testing our assumption required a longitudinal study to reveal the relationship between disease progression, occurrence of a pulmonary exacerbation and various components of a bacterial community, such as the change in diversity, richness, or the abundance of specific members of the microbiota."

"Most studies that you look at collect one pre-exacerbation sample, one during exacerbation and one during periods of stability," Stokell noted. "With that kind of study, it's hard to get an idea of how changes are occurring because those are more like cross-sectional analyses. Our focus was to look at how these changes are occurring generally, and in order to get some sort of baseline information we have to check changes frequently over time."

In discussing the work, Stokell is somewhat shy about mentioning that the unusually extensive sampling and analysis was only possible because of the willing contribution of an unusually cooperative patient: himself.

Because Stokell was both the researcher and the study subject, the team had unparalleled access to the subject's life history and medical records, providing critical context. "We have all kinds of patient background information," Stokell noted, "so we can associate the changes in bacteria with the occurrence of an exacerbation and also with when antibiotics are administered."

Chronic diseases like CF are often daunting puzzles that require an extraordinary amount of research effort to understand—the long-term

interaction between the human body's dynamic systems and the disease can be extremely complex, even when there's a relatively simple initiating cause, such as a genetic defect. Consequently, researchers studying such diseases need an extra amount of commitment and passion to struggle with the intricate complexities involved.

In Stokell's case, the commitment and passion came naturally because the disease is personal. UNC Charlotte biologist Todd Steck, whose lab Stokell now works in was Stokell's mentor, both in college and in graduate school. Steck observed that the experience of having cystic fibrosis played an important role in both developing Stokell's initial general interest in biology and in leading him to the line of research that he is now pursuing.

As a masters student at UNC Charlotte, Stokell took a class in bioinformatics from Anthony Fodor (also an author on the current paper), where students had to come up with projects involving metagenomic analysis of bacterial populations. Stokell recognized that this might be important research area relevant to his own disease, so he chose lung bacteria in cystic fibrosis, with himself as a research subject. Once engaged, he found that the interaction between CF and the human microbiome is difficult to unravel, but his personal connection to the disease kept him motivated.

The current study still does not answer all the questions raised by Stokell's class project, though he has now done both metagenomic analysis (which allows cataloging the full range of bacterial populations by finding variants of the 16S rRNA gene) and whole-genome sequencing (on a small group of bacterial varieties) on three year's worth of steady sampling. In addition to identifying *Burkholderia* as a key pathogen, the team found that richness and diversity of bacterial communities decreased over time, while the overall abundance of bacteria increased, perhaps due to ongoing antibiotic treatment and the

steady progress of the disease. However, those changes are not associated with the disease's damaging flare-ups.

"There were no changes in the metagenomic sequencing and the relative abundance of bacterial communities that were specifically associated with the occurrence of exacerbation," Stokell said. "Nothing that occurred within two weeks prior to the exacerbation indicated this is what is causing the individual to become ill.

"We did see treatment effects for richness and diversity - we saw those decreases during antibiotic treatment. And over a three-year period, bacteria continued to increase in abundance. But when we looked at specific pathogens, *Burkholderia* was the one that showed an increase in abundance prior to exacerbation. So we can speculate that that was the initiator of the exacerbation," he said.

The next step, the researchers say, is to do further study on a larger group of patients. Stokell and Steck have received grants from NIH and the Cystic Fibrosis Foundation that will allow them to do that. If findings in a larger sample support the pattern Stokell has found in himself, treatment of *Burkholderia* specifically may be an important target in managing the damaging effects of the disease.

Though significant research problems undoubtedly lie ahead, the real challenge for Stokell perhaps is the personal one he faces in the progress of his own disease. Though he has struggled all his life to maintain his health, at age 35 Stokell is now in late stage lung disease and will need a lung transplant sometime in the near future.

"I'm being considered for a double-lung transplant at Duke University Medical Center," Stokell said, explaining that this entails not just a complex operation but also three-months of pre-operation preparation and six months of recovery on-site, not to mention expensive

medications he will be required to take for the rest of his life. And, unfortunately, the difficulty of his situation is not limited to surviving until the transplant and enduring the medical realities.

"Undergoing a transplant is not only difficult because of the surgery, the associated monetary costs are quite high," he explained. "Typically, individuals in my situation are required to raise money prior to the surgery."

To raise the funds for transplant-related expenses, Stokell will be working with the Children's Organ Transplant Association (COTA), a 501(c)3 not-for-profit organization that receives donations to assist with transplant-related expenses. The goal for the COTA campaign in honor of Josh is $75,000.

With the cost of a transplant often exceeding $500,000, many transplant families are unable to shoulder the financial burden of such a procedure. The organization's priority is to ensure that a transplant does not financially devastate a family. One hundred percent of all funds raised are used for patients' transplant-related expenses.

"My hope is that I can spread the word about my need for a transplant and the financial needs I have associated with it," Stokell explained. "My transplant pulmonologist is guessing that I won't need a transplant for six months. However, because of the unpredictable nature of this disease at such a late-stage, that time could be quickly reduced or hopefully, extended."

Provided by University of North Carolina at Charlotte