# A shifting paradigm for cystic fibrosis screening in newborns

## Next-generation sequencing assays enable unbiased detection of pathogenic variants to maximize equity in screening

DR. MEI BAKER, MD,
UNIVERSITY OF WISCONSIN SCHOOL OF MEDICINE AND PUBLIC HEALTH

DR PHILIP FARRELL, MD, PHD
UNIVERSITY OF WISCONSIN SCHOOL OF MEDICINE AND PUBLIC HEALTH

Cystic fibrosis (CF) is a heritable autosomal recessive condition that affects multiple organ systems, leading to progressive lung injury and respiratory failure. Newborn screening is routinely used to identify infants with CF as early as possible so they can receive life-saving treatments that may prevent severe complications of CF. Most newborn screening programs use a genetic panel of pathogenic variants in the cystic fibrosis transmembrane conductance regulator (*CFTR)* gene frequently encountered in ancestral European and Ashkenazi Jewish populations. However, the limited scope of these panels can lead to missed diagnoses, lower risk reduction, and poorer outcomes in non-European populations.

Next-generation sequencing (NGS) offers a broader view into the CF-causing variants in the *CFTR* gene, minimizing demographic bias in existing genotyping panels. Dr Mei Baker and Dr Philip Farrell, at the University of Wisconsin School of Medicine and Public Health, have been instrumental in developing NGS-based algorithms to optimize CF screening and address disparities in current CF testing protocols. We spoke with Dr Baker and Dr Farrell about the importance of comprehensive newborn screening and the ways in which NGS-based testing is transforming patient care.

**Q: Why is newborn screening and early diagnosis important for inherited conditions like CF?**

**Philip Farrell (PF):** Diagnosis in the presymptomatic stage of a disease allows us to implement treatments that will be preventive. Newborn screening permits early diagnosis of CF, preventing deaths and morbidity, such as severe malnutrition and irreversible

lung disease. The value of early detection is essentially the same as for every genetic disorder on newborn screening panels. We believe every newborn should be screened for CF, particularly in regions where the incidence of CF is substantial. But even in regions like Tamil Nadu, India, where we think the incidence is much lower than in the United States, it still will be valuable because early diagnosis saves lives, so a pilot project is being planned there.

"Newborn screening permits early diagnosis of CF, preventing deaths and morbidity, such as severe malnutrition and irreversible lung disease."

**Q: Can you tell us about the current practices for CF screening in newborns?**

**Mei Baker (MB):** In general, for initial screening, laboratories use a serum biomarker called immunoreactive trypsinogen (IRT), which is an indicator of pancreatic disease that is commonly observed in CF patients. But not all pancreatic dysfunction is caused by CF, which can lead to false positive results. So, blood IRT is used as a first-tier test for screening. Most states use some form of genetic testing to identify *CFTR* variants as a second-tier test for newborn CF screening. A panel of *CFTR* variants is most commonly used. We believe the inclusion of all the known CF-causing variants on the panel is extremely important.

**Q: The American College of Medical Genetics recommends panels of 23 to 60 variants for prenatal CF screening. In your opinion, is this panel content sufficient?**

"There are so many possibilities of inequities, even when you do newborn screening. This is why comprehensive panels are very important to avoid disparities in care."

**PF**: No. The American College of Medical Genetics (ACMG) made that recommendation for population screening in pregnant women. Specifically, the panel of 23 mutations is for White women. That is not sufficient. This panel design itself has been discriminatory and leads to inequities. Consequently, the ACMG recently updated their recommendation encouraging the use of larger *CFTR* panels in newborn screening whenever possible.[1] There are so many possibilities of inequities, even when you do newborn screening. We know from data over the past 10 years that 10% to 20% of the babies diagnosed through newborn screening are not White.[2] These babies tend to be African American and Hispanic. The incidence is much lower in Asian Americans and Native Americans. You simply cannot be equitable with a panel of 23, or even the expanded panels with 39 or 60 variants. I think it's absolutely essential to achieve health equity and methods of diagnosis, like newborn screening, have the potential to enable that. This is why comprehensive panels are very important to avoid disparities in care.

**Q: How did you start using NGS for comprehensive newborn screening in your research?**

**MB**: CF is an autosomal recessive condition that occurs when an individual inherits two pathogenic *CFTR* variants. CF carriers are individuals who inherit a single pathogenic *CFTR* variant. When we got into the newborn CF screening field, we started with IRT testing and genetic testing with the 23 variants panel. Often, we were able to identify only a single CF-causing pathogenic variant, while the second contributing pathogenic variants were missed due to the limited number of variants on the panel. As a result, many true CF cases were misidentified as carriers. That is when it became clear to us that a larger panel was needed. With NGS, we could overcome the limitations of the conventional method. We could cover the whole gene potentially covering all known variants. However, deciding what variants to be included in the panel can be challenging. When we began using NGS assays for newborn screening, the Clinical and Functional Translation of *CFTR* (CFTR2) database became available. This is a great database for *CFTR* variants because it covers a larger population. I think now it includes data from about 90,000 patients and has identified over 400 pathogenic variants. Early on they also conducted some functional analysis on the variants.[3] So, you have the NGS technology available and this database to help you accurately identify variants. I think it was perfect timing.

" With NGS, we could overcome the limitations of the conventional screening method, that is, you could cover the whole gene, so you can potentially cover all the known variants. However, deciding what variants to be included in the panel can be challenging."

**PF**: It was the convergence of practical, affordable NGS, through Illumina, and the results of the CFTR2 project that facilitated the development of what we would call the IRT/NGS screening method.

"It was the convergence of practical, affordable NGS, through Illumina, and the results of the CFTR2 project that facilitated the development of what we would call the IRT/NGS screening method."

**Q: What are some of the concerns with using larger variant panels for screening?**

**MB:** The concern with large variant panels is that they may identify many more carriers than true CF cases. Theoretically, it's true, but practically, larger panels are not more challenging to work with to accurately identify CF cases. The reason is the common variants are already included in the 23-mutation panel, and many other mutations are familial. So, increasing the panel size gives screening programs a much better chance of identifying two variants in the true CF cases, and the increased number of carriers is much less than people are concerned about. We do not frequently encounter variants of unknown significance (VUS) because we now use a fixed panel with 372 pathogenic mutations for screening. These variants have already been defined as CF-causing in the CFTR2 database. That's our first pass, if you will. We do come across

what we call variants with varying clinical consequences, or VVCC. Sometimes, in addition to one known CF-causing variant, we find compound heterozygotes with a variant with varying clinical consequences, giving rise to a condition known as CF-related metabolic syndrome (CRMS). CRMS can evolve into CF, which is confirmed with an abnormal sweat chloride test, or SCT.

**Q: Can you tell us more about how you incorporate NGS into your newborn screening workflow?**

**MB**: The first step is the IRT screening test. When there is an elevated IRT result, the sample undergoes NGS using a comprehensive panel to identify variants. If one or two CF-causing variants are detected, a confirmatory sweat chloride test is ordered. If the sweat chloride results are 60 mmol/L or beyond, that confirms CF. However, if the sweat chloride results for single variant cases are 30 mmol/L or higher, we then reanalyze the variants. At this step, we do not use a panel and the analysis is open ended. This practice allows us to identify CRMS cases, including those that have the potential to become CF. This is what we call a staged analysis algorithm. This process identifies CRMS cases and also uncovers novel mutations not identified in the CFTR2 project.

**Q: Did you face any challenges when you first began using NGS in your research? How did you overcome them?**

**MB**: When we started to address comprehensive CF screening using NGS, we needed to make some modifications to the protocol to optimize DNA yield and concentration, especially because we use DNA from dried blood spots.[4] In the public health environment, when we screen many samples daily, we don't check DNA concentration, unlike in the clinical setting where we use a defined concentration of input DNA. For population-level screening, we do not have that much DNA available. Second, the sample-to-sample variability is very high. This is why it is essential to have a robust NGS assay that is tolerant of variations in DNA concentrations. Assay performance, stability, and reproducibility are crucial in the public health laboratory because every day we have new babies that need testing. We cannot afford backlogs.

**Q: How would the widespread adoption of NGS-based comprehensive newborn screening impact patient care?**

**MB**: Comprehensive testing is extremely important. If you can identify patients with CF in the first week, as well as identify the two causative variants, appropriate therapeutic decisions can be made quickly. This accelerates the whole treatment timeline.

"... it is essential to have a robust NGS assay that is tolerant of variations in DNA concentrations. Assay performance, stability, and reproducibility are crucial in the public health laboratory..."

" Comprehensive testing is extremely important. If you can identify patients with CF in the first week, as well as identify the two causative variants, appropriate therapeutic decisions can be made quickly."

Including NGS in our staged analysis also gives us the flexibility to identify new variants. Having analysis software allows you to rapidly identify the variants likely to be pathogenic. For example, in a case where the sweat chloride test is abnormal, you can go back and look for other variants. This approach not only allows us to find CRMS cases with the potential to become CF, but also identify novel variants for true CF patients. I would often say; Wisconsin is mostly White and we know all the variants because we've been screening for a long time. Turns out, I was wrong, because we found novel variants that have previously never been reported. In this case, I'm pleased to be wrong because we all learned something new!

"To have babies genotyped is very important because we now have *CFTR* modulators that can be used to treat cystic fibrosis in a highly effective way..."

**PF**: I will add that turnaround time is excellent with NGS assays. Most of the screening is completed on day 6, 7 or 8, in the vast majority of cases. If you can diagnose cystic fibrosis genetically from the dried blood spot specimen within one week, for 90% of the patients who have two pathogenic variants detected, that's almost miraculous! Not only have you made the genetic diagnosis from the dried blood spot, but you've also genotyped the patient. To have the babies genotyped is very important because we now have *CFTR* modulators that can be used to treat cystic fibrosis in a highly effective way, but we must know the genotype of the patient to use appropriate modulators.

**Q: Do you have any advice for laboratories that are planning to incorporate NGS-based assays in their screening workflows?**

**MB**: My advice is comprehensive newborn screening is important. I believe you should start with a panel that is inclusive and comprehensive. Take full advantage of the flexibility of the NGS analysis software, too. Be mindful of how you do the analysis, what you include in your results, and how you report the findings. Recognize the power of staged analysis that incorporates both, genetic and functional analysis markers. If you have abnormal sweat chloride values, you can reanalyze your variant results without the restrictions of the panel content. This will allow you to identify the true second variants for CF patients, and for the CRMS cases you intended to identify.

" My advice is comprehensive newborn screening is important. I believe you should start with a panel that is inclusive and comprehensive. Take full advantage of the flexibility of the NGS analysis software, too."

**Q: In addition to targeted NGS panels, is there a role for whole-genome sequencing (WGS) in newborn screening for CF?**

**PF**: I became interested in WGS about five years ago because it occurred to me that this approach might provide added value beyond early diagnosis through newborn screening.

With funding from an agency known as the Legacy of Angels Foundation, we have sequenced the genomes of about 180 CF patients to date. We have found it is reliable to extract small specimens of whole blood, using less than 1 ml of blood, harvest the white blood cells, extract the DNA, and perform WGS. For now, I think IRT/NGS is the optimum screening method. WGS, should only be used for children who have been diagnosed with CF and when looking for CF genetic modifiers. I would call it an ancillary test. Right now, it's premature to recommend WGS, but it's quite promising. I think we are at a stage now that we were in 2015 for newborn screening with NGS. It took about five or six years before we were satisfied with the IRT/NGS method. I think it's going to take another five years before we're in a situation where we might recommend WGS, which is becoming much more affordable.

**Q: What are your thoughts on the future of NGS in newborn testing and what role are you playing?**

**PF**: I think IRT/NGS will be the method of choice for CF newborn screening within a few years. Newborn screening labs are slow to change, but I also think a regionalization of NGS will be very important to make NGS-based screening the common screening method. There is a major initiative underway this year to improve the CF newborn screening algorithms in the United States. It is possible that the US Cystic Fibrosis Foundation, which is leading the initiative, is going to come out with a recommendation that newborn screening laboratories move as expeditiously as possible to expand *CFTR* panels, and NGS is the most appealing method to do so as we strive towards equity. History can help us here. After all states were screening newborns for CF in 2010 using IRT, it took 10 more years before all states were using a DNA-based screening method. Ten years is a long time when we knew, without a doubt, in 2010, that DNA-based methods were better than IRT. We now know that NGS is the superior method, but I think it's going to take at least five years for newborn screening labs to catch up.

**MB**: To follow what Dr. Farrell said, I think regionalization could potentially be part of the solution. I do believe that laboratories recognize the value of NGS-based testing, but part of the technical challenge is every state must be equipped to test using NGS platforms. Over time they will. I think the newborn screening paradigm may shift because we need, at least for now, both functional and genetic testing. Our staged analysis approach will help us understand all the variants and their clinical consequences. The way forward is to update the screening paradigm. Not only do you take care of the newborn in front of you, but also inform the future of newborn screening. We have to be forward thinking.

" Our staged analysis approach will help us understand all the variants and their clinical consequences. The way forward is to shift the paradigm. Not only do you take care of the newborn in front of you, but also inform the future of newborn screening. We have to be forward thinking."

## Learn more

NGS-based cystic fibrosis testing,
illumina.com/clinical/diagnostics/cystic_fibrosis

## References


1. Deignan JL, Astbury C, Cutting GR, et al. CFTR variant testing: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med.* 2020;22(8):1288-1295. doi:10.1038/s41436-020-0822-5
2. Patient Registry 2020 Annual Data Report. Cystic Fibrosis Foundation website. https://www.cff.org/media/23476/download. Accessed June 24, 2022.
3. Raraigh KS, Han ST, Davis E, et al. Functional Assays Are Essential for Interpretation of Missense Variants Associated with Variable Expressivity. *Am J Hum Genet*. 2018;102(6):1062-1077. doi:10.1016/j.ajhg.2018.04.003
4. Baker MW, Atkins AE, Cordovado SK, Hendrix M, Earley MC, Farrell PM. Improving newborn screening for cystic fibrosis using next-generation sequencing technology: a technical feasibility study. *Genet Med*. 2016;18(3):231-238. doi:10.1038/gim.2014.209



Drs Baker and Farrell report no conflicts of interest or personal financial relationships, including with Illumina.


illumina®


1.800.809.4566 toll-free (US) | +1.858.202.4566 tel | techsupport@illumina.com | www.illumina.com




M-GL-00740 v1.0