Researchers develop fast, accurate cystic fibrosis test

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Credit: AI-generated image

Researchers at the Stanford University School of Medicine have developed a fast, inexpensive and highly accurate test to screen newborns for cystic fibrosis. The new method detects virtually all mutations in the CF gene, preventing missed diagnoses that delay babies' ability to begin receiving essential treatment.

A paper describing the new test published online Feb. 1 in The *Journal of Molecular Diagnostics*. Cystic fibrosis, which causes mucus to build up in the lungs, pancreas and other organs, is the most common fatal genetic disease in the United States, affecting 30,000 people. To develop the disease, a child must inherit two mutated copies of the CF gene, one from each parent. Newborns in every U.S. state have been screened for CF since 2010, but the current tests have limitations.

"The assays in use are time-consuming and don't test the entire cystic fibrosis gene," said the study's senior author, Curt Scharfe, MD, PhD. "They don't tell the whole story." Scharfe was a senior scientist at the Stanford Genome Technology Center when the study was conducted and is now associate professor of genetics at the Yale School of Medicine.

"Cystic fibrosis newborn screening has shown us that early diagnosis really matters," said Iris Schrijver, MD, a co-author of the study and professor of pathology at Stanford. Schrijver directs the Stanford Molecular Pathology Laboratory, which has a contract with California for the state's newborn CF testing.

Advantages of early diagnosis, medical attention

Prior studies have shown that newborn screening and prompt medical follow-up reduce symptoms of CF such as lung infections, airway inflammation, digestive problems and growth delays. "When the disease is caught early, physicians can prevent some of its complications, and keep the patients in better shape longer," Schrijver said. Although classic CF still limits patients' life spans, many of those who receive good medical care now live into or beyond their 40s.

In the current test, babies' blood is first screened for immunoreactive trypsinogen, an enzyme that is elevated in CF cases but also can be high for other reasons, such as in infants with one mutated copy and one

normal copy of the CF gene. Since the majority of infants with high trypsinogen will not develop CF, most U.S. states follow up with genetic screening to detect mutations in the CF gene. California, which has the most comprehensive screening process, tests for 40 CF-causing mutations common in the state. (More than 2,000 mutations in the CF gene are known, though many are rare). If one of the common mutations is identified, the infant's entire CF gene is sequenced to try to confirm whether the baby has a second, less common CF mutation.

The process takes up to two weeks and can miss infants who carry two rare CF mutations, particularly in nonwhite populations about whose CF changes scientists have limited knowledge.

DNA from dried blood spots

The Stanford-developed method greatly improves the gene-sequencing portion of screening, comprehensively detecting CF-causing mutations in one step, at a lower cost and in about half the time now required. Stanford University is exploring the possibility of filing a patent for the technique.

To enable these improvements, the team developed a new way to extract and make many copies of the CF gene from a tiny sample of DNA—about 1 nanogram—from the dried blood spots that are collected on cards from babies for newborn screening. "These samples are a very limited and precious resource," Scharfe said. The entire CF gene then undergoes high-throughput sequencing. This is the first time scientists have found a way to reliably use dried blood spots for this type of sequencing for CF, which typically requires much more DNA.

"In our new assay, we are reading every letter in the book of the CF gene," Schrijver said. "Whatever mutations pop up, the technique should be able to identify. It's a very flexible approach."

In order for the new test to be adopted, the molecular pathology lab needs to train its staff on the new procedure and run thorough validation studies as part of regulatory and quality requirements to show that the reliability of the test in a research setting will be maintained in the larger-scale clinical laboratory. California newborn screening officials will then have the opportunity to decide whether they want the new test to replace the current method. Schrijver expects the process will take less than a year. "Regardless of how the state decides, the new technique can be widely adopted in different settings," she said, noting that the technique could also be used for carrier and diagnostic testing and to screen for other genetic diseases, not just CF.

"Ultimately, we would like to develop a broader assay to include the most common and most troublesome newborn conditions, and be able to do the screening much faster, more comprehensively and much more cheaply," Scharfe said.

The work is an example of Stanford Medicine's focus on precision health, the goal of which is to anticipate and prevent disease in the healthy and precisely diagnose and treat disease in the ill.

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