Cystic Fibrosis Screen-Positive Neonates with One Pathogenic Variant Still Warrant Sweat Testing

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Abstract

We believe that the data in this letter clearly demonstrate that even with CFTR2 expansion to 719 variants, striving to achieve equity of early diagnosis of CF via screening requires states to perform a sweat test in all infants with a high IRT level and one identified *CFTR* variant. This recommended policy can be debated but sweat testing overload should not be the argued as the barrier and CF specialists need to recognize that CFTR2 may never include all of the very rare, "private" pathogenic variants nor will next generation sequencing cover the structural variants such as deletions and duplications.

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To the Editor,

Many challenges remain as states seek to improve newborn screening (NBS) for cystic fibrosis (CF), especially to achieve the goals of equity and timeliness. Controversies abound with regard to procedures (NBS algorithms such as the size of panels to identify variants in the cystic fibrosis transmembrane conductance regulator or *CFTR* gene), practices (operations), and policies (e.g., selecting babies for follow-up diagnostic evaluations). Our recent article[1]entitled "Refinement of newborn screening for cystic fibrosis with next generation sequencing" described the evolution of the NBS algorithm in Wisconsin to maximize detection of infants with CF through an expanded*CFTR* panel achieved with next generation screening methodology and updateable reporting software. In brief, we utilized the CFTR2 database to screen for CF-causing variants in infants who had an elevated immunoreactive trypsinogen level (with a floating cut-off at the 96^{th} percentile). The number of CF-causing variants is increasing over time as we learn more about the disease liability of different *CFTR* variants. The CFTR2 project is an international effort to characterize variants as CF-causing (pathogenic), non-CF-causing, variants of varying clinical consequences (VVCCs) and variants of unknown significance. Utilizing the CFTR2 database was a cornerstone of our refinement of NBS.

In phase 2 of our NBS studies, we utilized variant lists from CFTR2 that were published in 2013 and 2016. In phase 3, we utilized four variant lists published in CFTR2, with the most recent being CFTR2_-24September2021.xlsx (variant lists from https://www.cftr2.org/mutations_history). This variant list has 382 variants that are CF-causing. When we published our article,[1] there were a total of four infants diagnosed with CF in phase 2 plus phase 3 who had novel variants not in CFTR2. (The original published article stated that there were six infants who had novel variants that were not in CFTR2. Due to typographical errors in the legacy name of two variants, the correct number of infants with novel variants is four.)

Since then, there are two more recent variant lists in CFTR2. The variant list CFTR2_29April2022.xlsx has 401 CF-causing variants and CFTR2_7April2023.xlsx includes 719. If we had used this most recent variant list, we would still have identified the same four infants with CF who each have one detected variant and a novel variant not in CFTR2. To avoid or at least minimize false negatives and strive for equity, we have

always recommended a sweat test for all infants born in Wisconsin who have one pathogenic variant, so these infants were diagnosed at an early age.

McGarry et al[2] utilized the CF Foundation Patient Registry to analyze the detection of 1 or 2 variants using variant panels of different size (ranging from F508del only to the 401 pathogenic variants in CFTR2 from CFTR2_29April2022.xlsx). When this*CFTR* 2 database was utilized, detection of one variant was lowest in people who were Asian, non-Hispanic (77.4%), and detection of two variants was particularly low in Asian, non-Hispanic (53.9%), Hispanic (73.7%) and mixed races, non-Hispanic (76.0%). With CFTR2 now being updated to 719 pathogenic variants (CFTR2_7April2023.xlsx), we asked Alex Ebert and Runyu Wu to reanalyze the data in the McGarry article with this most recent updated variant list. Tables 1 and 2 include the last two lines from the data in the McGarry et al[2]publication (CFTR2_22April2022.xlsx which has 401 CF-causing variants), and an additional two lines in each table utilizing the 719 CF-causing variants in CFTR2_7April2023.xlsx alone, and 719 CF-causing variants plus VVCCs. Of note is that there is only a minimal increase in the detection of 1 or 2 variants in all races/ethnicities with this most recent larger variant panel. Specifically, detection of one variant in Asian, non-Hispanic people with CF only increased from 77.4% to 80.6%, and detection of two variants also increased minimally (Asian, non-Hispanic: 53.9% to 56.2%; Hispanic: 73.7% to 75.9%; and mixed races, non-Hispanic: 76% to 78.4%).

We concluded our article[1] with a policy assertion "that all infants with one variant should have sweat testing performed." Even though CFTR2 has added 318 more pathogenic variants, if we had not performed a sweat test in all 1-variant infants, we would have missed diagnosing CF in 5% of the infants in Wisconsin in phase 2 plus phase 3 of our improved NBS program. Although this proportion of missed cases is better than the 11% during 2010-2018 nationally,[2] preventable false negative results should obviously be avoided. These data are even more compelling in looking at the updated analysis from the McGarry et al[2]publication, particularly in people of color or minority groups. Requiring that 2-variants be present to proceed to sweat chloride determination would result in *not* establishing an early diagnosis in 43.8% of Asian, non-Hispanic patients, 37.3% of African American/Black non-Hispanic patients and 14.1% of Hispanic patients. But continuing to expand *CFTR* panels is important to strive for equity. Thus, Wisconsin's panel now includes 689 pathogenic variants from the CFTR2 update.

Although there are several arguments of not performing sweat testing in infants with one variant and arguments against increasing the size of*CFTR* variant panels, we do not believe that those arguments are compelling, particularly in the emerging era of next generation sequencing. A list of misperceptions and counterarguments related to policies follow:

- *Sweat test labs could be overwhelmed with the number of sweat tests* . Prior to CF NBS, providers typically ordered sweat tests for children with gastrointestinal or respiratory symptoms. With routine NBS, providers now order many fewer sweat tests. We have documented[3] that the number of sweat tests decreased in Wisconsin with the introduction of CF NBS from 1670 to 804 (including 134 follow-up tests from screening). Thus, in the NBS era, sweat test labs are performing many fewer sweat tests compared to pre-newborn screening but need to perform enough annually to maintain proficiency.
- *Sweat testing infants will result in unacceptably elevated quantity not sufficient (QNS) sweat collections.* It is true that there are challenges in obtaining sufficient sweat from young infants.[4] Factors such as low birth weight and gestational age can have impacts on obtaining an adequate quantity of sweat. However, the CF Foundation has committed to improved oversight and performance of sweat testing in CF Centers. The site visit process includes an in-depth examination of sweat collection and analysis, with scrutiny of the protocol by the Sweat Test Advisory Committee. Additionally, individual centers can engage in quality improvement activities to decrease their sweat test QNS rate.[4]Even if there is a QNS sweat collection, genetic counseling delivered at the time of the sweat test is a benefit that most parents appreciate.
- *The Illumina MiSeqDx next generation sequencing assay is only approved for 139 variants.* The FDA approval for the 139 variant method applies only to blood specimens. We performed a full validation of using this technology on dried blood spots.[5] The variant calling file can be updated through the

reporting software as needed to add more variants when CFTR2 is updated. Variants that are not the FDA approved 139 variants can be verified via Sanger sequencing. We include a statement on our screening results reports to providers as follows: "These tests have not been cleared or approved by the FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. These tests are used for clinical purposes and should not be regarded as investigational or for research."

- *The next generation sequencing assay is too time consuming.* We demonstrated that our timeliness benchmarks (6-9 days) were being met with next generation sequencing.[1]
- *It is too costly to increase the number of CFTR variants.* The cost is no different for 139, 401 or more variants. The variant calling file can be readily updated with each expansion of CFTR2 variant as we have done. And our current costs of analysis for 689 variants are the same as the cost of analysis for 401 variants.

There is documentation showing that historically marginalized groups have delayed entry into the health care system and worse nutritional outcomes.[6] As it stands now, early diagnosis of CF via newborn screening depends on where and when a baby is born. An African/American or Hispanic or Asian infant born in Wisconsin has the same likelihood to be diagnosed early via newborn screening. If that same baby was born in states that perform sweat testing only if 2 variants are identified, a practice introduced in California, there is a much higher chance that the 1-variant infant will have a delayed diagnosis. Thus, those protocols are not equitable across all races and ethnicities. We believe that the data in this letter clearly demonstrate that even with CFTR2 expansion to 719 variants, striving to achieve equity of early diagnosis of CF via screening requires states to perform a sweat test in all infants with a high IRT level and one identified *CFTR* variant. This recommended policy can be debated but sweat testing overload should not be the argued as the barrier and CF specialists need to recognize that CFTR2 may never include all of the very rare, "private" pathogenic variants nor will next generation sequencing cover the structural variants such as deletions and duplications.

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`Tables 1 and 2.docx` available at https://authorea.com/users/475154/articles/654061-cystic-fibrosis-screen-positive-neonates-with-one-pathogenic-variant-still-warrant-sweat-testing