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Cystic fibrosis diagnosed by state newborn screening: Or is it?

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Abstract

Newborn screening for cystic fibrosis is universal across the United States; however, each state chooses the method by which they screen. Illinois employs a two-step process which includes the measurement of the immunoreactive trypsinogen followed by an assay designed to detect 74 of the most common genetic mutations in the cystic fibrosis transmembrane conductance regulator protein. We report the case of an infant born in Illinois with a positive cystic fibrosis newborn screening with an elevated immunoreactive trypsinogen and two genetic mutations identified (F508del/F508del). The primary care physician informed the parents their child had cystic fibrosis and referred her for a confirmatory sweat test which was negative for cystic fibrosis. Upon further investigation, the assay was found to have been set up incorrectly and repeat analysis identified the genotype F508del/F508C. This case highlights the importance of performing the confirmatory sweat test prior to making a diagnosis of cystic fibrosis.

Keywords

Cystic fibrosis, newborn screen, pediatrics, genetic counseling

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Introduction

Cystic fibrosis (CF) is the most common life-shortening autosomal recessive disease in Caucasians, affecting ~30,000 people in the United States.[1] CF is caused by mutations in the gene that codes for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, responsible for chloride and bicarbonate transport across epithelial cell surfaces. Abnormal chloride transport through the CFTR leads to clinical problems such as pancreatic insufficiency, severe malnutrition and malabsorption, fat-soluble vitamin deficiencies, and progressive obstructive lung disease.[2]

Improved nutritional outcomes are associated with early detection and initiation of treatment in CF.[3,4] Inclusion of CF in state newborn screening (NBS) programs has increased the ability to diagnose CF during infancy, with all 50 states performing the CF NBS as of 2010.[5] In Illinois, the measurement of the immunoreactive trypsinogen (IRT) level by fluorometric assay is the first tier of the CF NBS. If the IRT is in the top 4% of the day or qualifies as an ultra-high IRT of $\geqslant$170 ng/mL, second tier DNA mutation analysis for a panel of 74 CFTR mutations is performed.[6] A positive CF NBS is defined as (1) an elevated IRT and the presence of one or two CFTR mutations, or (2) an ultra-high IRT level with no mutations.[7] Positive screens must be followed by a confirmatory quantitative pilocarpine iontophoresis sweat test done at an accredited CF center.[6,8] A chloride concentration of $\geqslant$60 mmol/L is diagnostic for CF.[7]

The nature of the CF NBS process creates an inherent risk of false-positive (FP) results. The majority of positive CF NBS identify carriers only; however, this delineation is not made until the confirmatory sweat test is performed. Short-term anxiety and feelings of depression have been reported among parents awaiting definitive diagnostic assessment.[9] The speed with which a confirmatory sweat test is performed,

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as well as communication and education from specialized CF centers, can help reduce parental anxiety.[10] A knowledge gap not included in previously published work is the possibility of an error in the NBS test itself. This case report describes a FP diagnosis of CF made through the incorrect identification of two CFTR mutations (F508del/F508del) by the Illinois State Department of Health NBS Laboratory.

Case report

Our institution does not require ethical approval for reporting individual cases or case series. Written informed consent was obtained retrospectively from a parent for the anonymized patient information to be published given the subject is a minor. A 28-day-old female term infant born in May 2018 with a positive Illinois State CF NBS was referred to Ann & Robert H. Lurie Children's Hospital of Chicago for genetics consultation and confirmatory sweat testing. The NBS report revealed an IRT of 51.2 ng/mL (top 4% of day) and two CFTR mutations (F508del/F508del) identified by Agena Bioscience massARRAY® CF assay. Based on this result, the community physician informed the family that their daughter had a new diagnosis of CF. The parents were instructed to call Lurie Children's Hospital for a diagnostic sweat test and further management.

The CF NBS process at Lurie Children's Hospital consists of an initial consultation with a genetics counselor to gather demographic and clinical information and to schedule the sweat test. During this phone consult, the parents expressed disbelief regarding the CF diagnosis given that during the pregnancy they each completed "23andMe" home genetic testing that showed the father to be a carrier of the F508del mutation; however, the mother did not have any CFTR mutations identified. 23andMe is a Personal Genetic Service that uses qualitative genotyping to detect select clinically relevant variants in the genomic DNA of adults from saliva. Given the presence of the CFTR genotype F508del/F508del on CF NBS, a true CF diagnosis was still assumed and a clinic appointment with the CF team was scheduled in addition to the confirmatory sweat test.

Hospital course and final diagnosis

Confirmatory sweat chloride testing done via the Macroduct Sweat Collection System and following Cystic Fibrosis Foundation (CFF) and Clinical and Laboratory Standards Institute (CLSI) Guidelines[7,8] was normal (10 mmol/L and 11 mmol/L, adequate volume of >15 µL collected; normal <30 mmol/L). In brief, stimulation of sweat using pilocarpine iontophoresis for no greater than 30 min is required, followed by collection of sweat into gauze, filter paper, or Macroduct coils. An adequate volume (microliters) or weight (milligrams) of sweat must be confirmed or the specimen will be identified as quantity not sufficient (QNS). Chloride concentration is then measured quantitatively using a chloridometer. The test should be performed in duplicate, and only called QNS if both samples are below recommended volumes.

Combining the lack of the CFTR mutation F508del in the mother plus the negative sweat test in the infant suggested that the CF NBS results were erroneous. Parents were appropriately tearful, as they had been previously told their infant had CF. Based on the sweat test result, the diagnosis of CF was likely a mistake.

Upon discussion with the Illinois State Health Department Newborn Screening Laboratory, it was determined that the assay used for detecting CFTR mutations in newborn blood spots had been incorrectly set up when a new testing platform was introduced earlier in the year. The archived blood spot for this infant was re-tested, and she was found to have F508del/F508C. Given that F508C is a benign polymorphism, she was correctly identified as a carrier of one CFTR mutation (F508del) and did not have CF.

Discussion

CF NBS programs were designed to identify infants with CF before they become symptomatic. Prompt follow-up diagnostic sweat testing can lead to earlier treatment, which can have significant health benefits for patients compared with those diagnosed at a later age.[3–5] Although the benefits of NBS are well documented, this case study highlights the importance of a strong relationship with the state newborn screening lab and the importance of a confirmatory sweat test before a true CF diagnosis can be made.

An open line of communication between CF centers and state laboratories is key to address confusing situations as rapidly as possible. It is the state's responsibility to oversee key aspects of NBS including initial screening, confirmation of diagnosis, and coordination of follow-up for infants with out-of-range screening results.[11] It is also the state's responsibility to maintain up-to-date technology and have clearly delineated collection and handling procedures. The incorrect identification of this 28-day-old infant as F508del/F508del is just one example of what can occur as state laboratories update and make changes to their screening platforms. Our CF center noted the discordance between the NBS result, the sweat test, and the parental history which prompted a quick investigation and resolution of the incorrect CF NBS report. A new testing platform had been implemented earlier in the year and it was determined that F508C, along with other benign polymorphisms, had been "turned off" and was no longer able to be detected. This change was made to prevent the confusion created by reporting benign polymorphisms in state NBS reports. In brief, the state lab uses the Agena Bioscience massARRAY® system which produces a spectrum with unique peaks for each CFTR mutation. Using F508del as an example, both a wild type (WT) and mutation signature can be generated, and the instrument will detect which one is present in a patient blood spot sample. Turning

off benign polymorphisms creates a situation where no WT peaks are seen, "tricking" the instrument into thinking two copies of the F508del mutation were present. The state lab repeated the assay on the patient's archived blood spot with the benign polymorphisms "turned on" and correctly identified the infant as F508del/F508C. This case led to the implementation of a confirmatory test with the polymorphisms "turned on" when infants with two CFTR mutations are found. The state retroactively searched for other infants reported to be F508del/F508del since the new platform assay was initiated and found no additional cases.

This case also emphasizes the importance of performing the gold standard, confirmatory sweat test prior to determining a true CF diagnosis. The CF NBS is a screening test and a positive result needs to be confirmed. The CFF recommends a sweat test be performed within 1 month of life in all infants with a diagnosis of CF, even those with two CFTR mutations identified by NBS. In this case, the parental genetic testing and the failure to identify both parents as carriers raised the suspicion for a FP NBS; however, this was not clarified until the sweat test was resulted as normal. All infants with a positive state CF NBS should be referred to an accredited CF Center as quickly as possible to have this confirmatory sweat test performed.

Finally, this case also brings up the question about what emotional impact direct-to-consumer genetic testing has. With the rise of "home genetic testing kits," there are questions about shifting control of genetic testing to consumers versus medical professionals. Harm can fall on the consumers who fail to understand the significance of the information provided or worry unnecessarily about the significance of results.[12] Although the "23andMe" results played a key role in identifying the discrepancy in the NBS, the discussion about the results still needed the appropriate medical professionals and genetic counselors. As genetic information becomes more readily available outside of the medical home, the impact of this information on families should be considered.

Conclusions

This case study demonstrates that methodological errors in CF NBS can occur, emphasizing the importance of the sweat test to confirm CF. A direct line of communication is of utmost importance as well: between the CF center and genetic counselors after positive NBS to alleviate parental anxiety, as well as between the state lab and CF center for quick resolution of discrepancies.

Author contributions

M.F. contributed to design, contributed to acquisition, drafted manuscript, critically revised manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy; A.M. contributed to conception and design, contributed to analysis and interpretation, critically revised manuscript, critically revised manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy; A.S. contributed to conception and design, contributed to analysis and interpretation, critically revised manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy; T.A.L. contributed to conception and design, contributed to acquisition, analysis, and interpretation, critically revised manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

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Ethical approval

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Informed consent

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article. The patient is a minor, so consent was obtained from a parent. Dr. Laguna has an email written by both parents of the minor who is the subject of the case report providing permission to publish this anonymized work.

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