diagnosed with CRMS may be followed by CF providers or general pulmonologists. European guidelines published in 2021 [1] precipitated an effort to standardize care across providers at our large CF center, as well as in general pulmonology groups. This quality initiative developed a standard CRMS care pathway based on guidelines and provider preference and established a CRMS clinic.

Methods: A survey about CRMS management was distributed to all pediatric CF providers. The survey consisted of seven multiple choice and one open-response question. Questions focused on provider preference for management such as follow-up timeline, throat and sputum cultures, and age of transition.

Results: Survey data revealed discrepant preferences across providers for CRMS management. Most providers reported that they would refer to a CRMS clinic run by an advanced practice provider that also has a genetic counselor present for annual management following established diagnosis of CRMS. Time of referral was split, with 22% choosing to refer immediately after diagnosis, 44% after 6 months old, and 33% after 2 years of age. Preference for throat cultures was split at 50%. Transition from CRMS clinic to as needed rather than annually was divided: 20% at aged 6, 40% at aged 14 to 16, and 40% at age18 with transition to adult CF center. Results of the survey were reviewed at a clinic-wide quality improvement meeting, and a final care pathway flow-chart was created and distributed to all providers as a reference for a new standardized care pathway.

Conclusions: This quality improvement initiative aimed to optimize consistency of care for patients with CRMS. Results of the survey emphasized that provider preference varies. Based on these findings and guideline implementation, our CF center has created a biannual CRMS clinic managed by advanced practice providers and a CF genetic counselor. The distributed flow-chart provides standard management for providers before referral to an annual CRMS clinic. In open-ended responses, providers emphasized inclusion of a genetic counselor in these clinics, which will provide opportunity for regular variant review, updates on variant classification, and counseling for families, as needed.

Reference

[1] Barben J, Castellani C, Munck A, Davies JC, de Winter-de Groot KM, Gartner S, *et al.* (ECFS NSWG). Updated guidance on the management of children with cystic fibrosis transmembrane conductance regulator-related metabolic syndrome/cystic fibrosis screen positive, inconclusive diagnosis (CRMS/CFSPID). *J Cyst Fibros* 2021;20(5):810–9.

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Variable genetic counseling access and services for parents of infants who screen positive for cystic fibrosis in New York State

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Background: The Cystic Fibrosis Foundation (CFF) has endorsed guidelines recommending genetic counseling by a provider trained in genetics with expertise in CF for all families of infants with a positive cystic fibrosis (CF) newborn screening (CFNBS) result [1]. The New York State (NYS) CFNBS Consortium has spearheaded an ongoing quality improvement effort to characterize the processes of the NYS CFNBS program and CF specialty care centers (SCCs), with a goal of improving outcomes and increasing access to care for referred infants with abnormal CFNBS results. NYS uses a three-tier immunoreactive trypsinogen (IRT)-deoxyribonucleic acid (DNA)-sequencing (SEQ) algorithm for CFNBS; infants with two or more CFTR variants of potential clinical relevance are referred for follow-up to an SCC. Infants with one CFTR variant are not referred to an SCC. Because the NYS Department of Health reports complex genetic information that requires interpretation and counseling about its implications, we sought to establish baseline rates of genetic counseling provided to parents of infants with CF screen-positive, inconclusive diagnosis/CF transmembrane conductance regulator (CFTR)-related metabolic syndrome (CFSPID/CRMS) or CF carrier status.

Methods: As part of a longer-term follow-up effort by the NYS NBS program and SCCs, a clinical and demographic dataset was compiled for infants referred between December 1, 2017, and November 30, 2021. Genotypes and CFTR phasing data were abstracted from NBS records. Demographic and clinical data, including information about provision of genetic counseling services, were requested from each SCC and tabulated to assess genetic counseling rates. Infants with a positive CFNBS (high IRT and more than one CFTR variant, with one or more variants of uncertain significance or varying clinical consequence), were included in this analysis. Infants classified as having CF were excluded. The NYS Department of Health Institutional Review Board determined this project to be exempt.

Results: Of 290 infants meeting study criteria, 273 (94.1%) were evaluated at least once. Of those, 225 were subsequently classified as having CFSPID/CRMS or as CF unlikely or CF carrier. Parental phasing studies confirmed that all CFTR variants were in cis for 32 infants, or the second variant was reclassified as non-CF-causing (n = 16). SCCs reported that 97 of 273 (35.5%) families saw a trained genetic counselor. CF physicians provided genetic counseling to 51 families (18.7%). Genetic counseling was not provided to 79 families (28.9%); data were not reported for 46 families (16.8%). Reasons provided for lack of genetic counseling included that a genetic counselor was not available (n = 15), the family already had knowledge because of prior child or prenatal testing (n = 3), the infant was adopted or in foster care (n = 2), and the family declined (n = 9). Genetic counseling rates at NYS CF centers for this population ranged from 0% to 100%.

Conclusions: Genetic counseling is provided inconsistently to parents of infants with a positive CFNBS result in NYS; in nearly one-third of cases in which genetic counseling was noted, the CF physician provided it. Inaccessibility of a genetic counselor was the primary reason given when genetic counseling was not performed. Additional efforts are needed to address access barriers to implement CFF recommendations regarding genetic counseling for parents of infants with a positive CFNBS result.

Reference

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My patient has an unresolved *CFTR* genotype…what next?

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Background: Most individuals with cystic fibrosis (CF) have two causal variants in separate CF transmembrane conductance regulator (*CFTR*) alleles identified via genetic testing using a variant panel or sequencing of

the coding and flanking intronic regions. Despite improved genetic testing technology and greater understanding of rare *CFTR* variants, a small subset of those with a clinical diagnosis of CF are left with an incomplete genotype —0 or 1 identified causal variant—and thus have uncertainty regarding prognosis, treatment, and genetic counseling. We sought to consider options for such cases, report on retrospective and prospective in-depth genetic analysis, and suggest next steps for evaluation.
Methods: A randomly selected subset of individuals with sweat chloride greater than 60 mmol/L enrolled in the Cystic Fibrosis Foundation Mutation Analysis Program (MAP; n = 4479 total enrollees) who had undergone exonic *CFTR* sequencing and deletion–duplication analysis that identified no (n = 20) or one (n = 20) causal *CFTR* variants, underwent full-gene sequencing of *CFTR*. Prospective analysis included upstream, downstream, and intronic regions. Forty-nine individuals with CF from the CF Genome Project (CFGP; n = 5058, 0.97% of the cohort) who had undergone whole-genome sequencing (WGS) with no or one causal *CFTR* variant identified underwent retrospective phenotype review, investigation of noncoding regions of *CFTR,* and interrogation of other genes. We searched for loss-of-function variants in all protein-coding genes and screened 866 genes implicated in CF-like phenotypes, the CFTR interactome, or CFTR biogenesis and functional pathways.
Results: After full-gene *CFTR* sequencing, nine of 20 individuals (45%) from MAP with one known causal variant had a second putatively causal variant identified: six intronic (pathogenic: c.1680-877G >T, c.3874-4522A >G; likely pathogenic: c.2909-15 T >G, c.3469-1304C >G; VUS: c.1584+689G >A, c.2490+1083C >T) and one pathogenic partial exon deletion (c.3325_3367 +249del). No putatively causal variants were found in those with no previously identified causal *CFTR* variants. Of 49 CFGP subjects considered, 43 were excluded because their *CFTR* genotypes contained a non-CF-causing variant previously considered causal (n = 24), WGS did not confirm the genotype reported in clinical records was (n = 5), or a CF phenotype was not confirmed (n = 14). Of the six remaining subjects, siblings (n = 2, 33%) with one known causal *CFTR* variant were found to have a putatively causal deep intronic variant (c.3874-4522A >G); no variants of interest were detected in the other four individuals. Screening of other genes revealed no other variants that would explain these individuals' phenotypes.
Conclusions: Individuals with a clinical diagnosis of CF and one known causal variant may benefit from full-gene *CFTR* sequencing, which can detect deep intronic and complex structural variation. Individuals with no causal variants after typical clinical testing are unlikely to benefit from further interrogation of *CFTR*, although ancestry or consanguinity may affect decision-making regarding further testing. A small fraction of those with clinical features of CF and an incomplete genotype may be symptomatic CF carriers, epigenetic cases, or phenocopies. Whole-genome long-read sequencing may provide further insight. The large number of individuals in the CFGP excluded (43/49) suggests that clinicians should frequently review updated interpretations of *CFTR* variants and consider whether a patient's phenotype remains consistent with CF.

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Improving consistency of examination for infants with positive newborn screening and inconclusive diagnosis

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Background: Newborn screening (NBS) for cystic fibrosis (CF) has led to a rise in the number of infants with positive NBS but inconclusive diagnosis (Figure 1). Despite availability of guidelines for the examination and management of infants with suspected CF transmembrane conductance regulator (CFTR)-related metabolic syndrome (CRMS), rapid advances in genetic testing have resulted in variations in practice at our center. To address these discrepancies, Children's Healthcare of Atlanta and the Emory University Pediatric CF Program started a quality initiative to establish institutional guidelines for infants with positive CF NBS, intermediate sweat test, and uncertain genetic testing.
Methods: A six-question survey about management of infants with inconclusive diagnoses was sent to all pediatric CF providers: three multiple choice questions and two multistep diagnostic scenario questions.
Results: Survey data identified discrepancies in provider examination of infants with positive NBS and intermediate sweat test. Some providers preferred to wait for repeat sweat test, whereas others chose to pursue genetic testing immediately. Results were reviewed at a clinic-wide quality improvement meeting. Key cases were discussed, including an infant found to have two CFTR variants whose sweat test normalized by 2 months and thus could have been falsely cleared as a carrier without early sequencing and an infant whose sequencing was delayed and was eligible to start ivacaftor before testing. A guideline for infants with positive NBS and uncertain diagnosis was developed to ensure consistency of all providers. The algorithm recommends CFTR sequencing and deletion–duplication testing after initial intermediate sweat test, as well as a consistent follow-up schedule for CRMS patients.
Conclusions: This quality improvement initiative was designed to optimize quality and consistency of care for infants with positive CF NBS, intermediate sweat testing, and uncertain genetic testing. We plan to follow up after algorithm implementation to determine effectiveness. During the development process, providers noted the critical benefit of having a genetic counselor experienced in CF involved in the diagnostic process to assist with interpretation of genetic results and provide counseling to families.

CLINICAL TRIALS & OUTCOME MEASURES

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In vitro responses of F508DEL human nasal epithelial cells correlate with clinical improvement with elexacaftor/tezacaftor/ivacaftor

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Background: Well-differentiated human airway epithelial cells (HAECs) have formed an important basis for preclinical evaluation of cystic fibrosis (CF) transmembrane conductance regulator (CFTR) modulators. Advances in expansion of cells have been leveraged through multisite collections of human nasal epithelial cells (HNECs) through Cystic Fibrosis Foundation Therapeutics Development Network–sponsored observational studies. Our lab has demonstrated that in vitro effects of ivacaftor in G551D HNECs—specifically CFTR function and airway surface liquid (ASL) or mucociliary transport (MCT) rate—correlated with clinical improvements in sweat chloride and lung function (percentage predicted forced expiratory volume in 1 second (FEV_1pp)), respectively, in the same patients in response to ivacaftor during the Gaining Optimal Asthma Control (GOAL) study, which was designed to determine whether HNECs derived from patients respond to elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA) and whether the functional responses correlate with clinical improvement with newly initiated ELX/TEZ/IVA on a groupwise and individual basis in the Prospective Study to Evaluate Biological and Clinical Effects of Significantly Corrected CFTR Function (PROMISE study).
Methods: HNECs were collected from donors participating in the PROMISE study and expanded using conditional reprogramming. Cells were differentiated at the air-liquid interface using Ultroser-G–supplemented differentiation media for 21 to 28 days. Inserts were analyzed for forskolin-stimulated CFTR-dependent current (Isc) using modified Ussing chambers after pre-treatment with elexacaftor (3μM), tezacaftor (3μM), and ivacaftor (acute treatment, 10μM) followed by CFTR Inh-172 (20 μM). Each donor was analyzed for change in MCT rate, ASL depth, and ciliary beat frequency (CBF) using micro-optical coherence tomography after chronic (48-hour) treatment with ELX/TEZ/IVA.
Results: Differentiated monolayers from 19 donors (10 F508del/F508del, 9 F508del/minimal function) have been analyzed. Monolayers demonstrated a mean ELX/TEZ/IVA-treated, forskolin-stimulated Isc of 7.6 ± 3.0 μA/cm^2