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

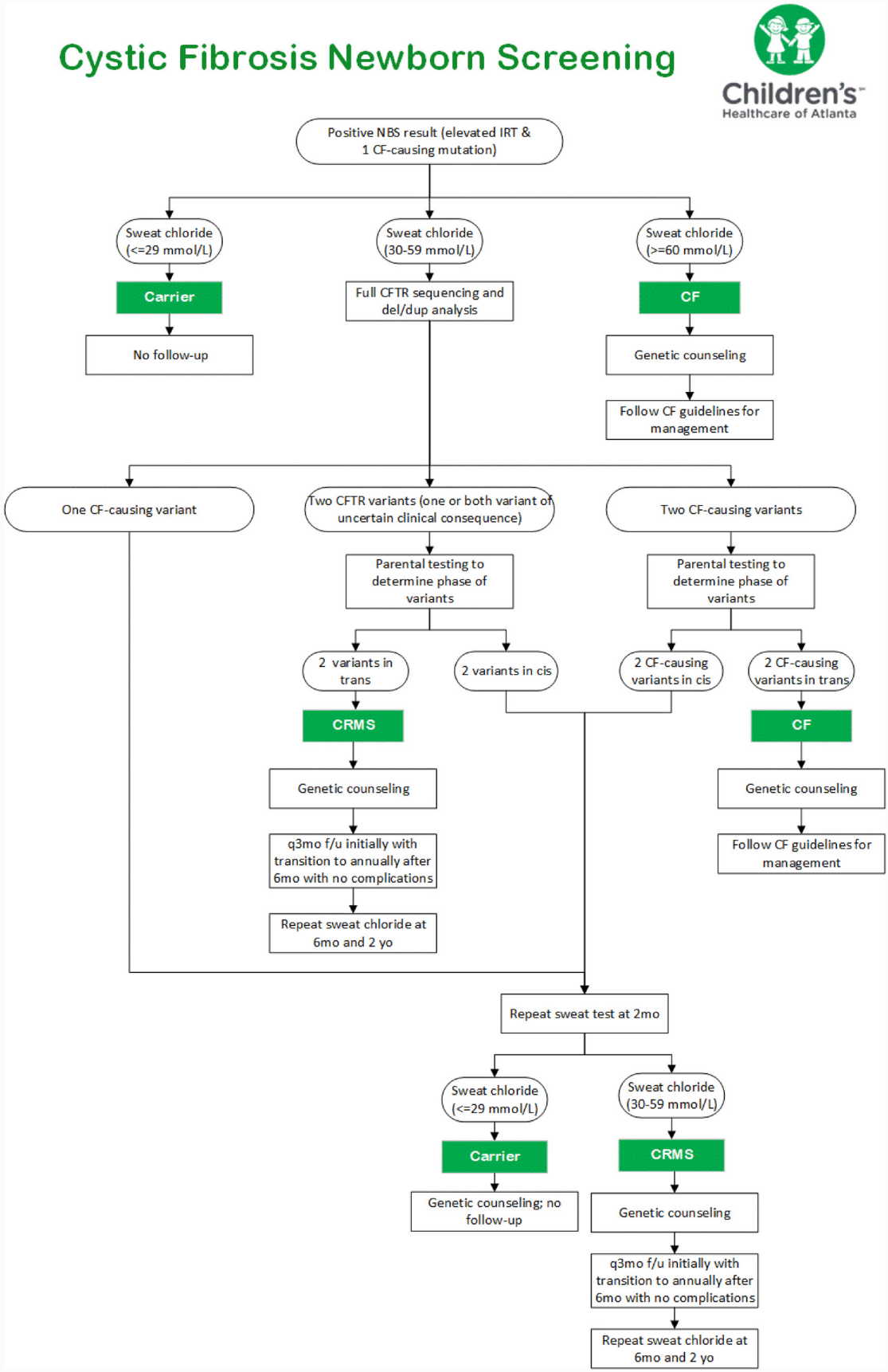

Figure 1 (abstract 153): Standardized flow-chart for newborn screening genetic testing.

(vs 2.1 ± 2.0 μA/cm^2 for vehicle, $p < 0.001$, representing 48% restoration of wild-type CFTR Isc). CFTR inhibition with CFTR Inh-172 mirrored these responses. Monolayers demonstrated improved ASL (11.9 ± 4.9 μm ELX/TEZ/IVA vs 5.3 ± 2.5 μm vehicle control, $p < 0.05$, 56% improvement with ELX/TEZ/IVA vs vehicle normalized to baseline measurements); acquisition of MCT and CBF data was limited because of variable degrees of differentiation. On an individual basis, ELX/TEZ/IVA-treated Fsk-stimulated Isc correlated with augmented ASL with ELX/TEZ/IVA treatment (r = 0.74, $p < 0.001$). ELX/TEZ/IVA-treated Isc also moderately correlated with 6-month post-ELX/TEZ/IVA sweat chloride (r = 0.59, $p < 0.05$) but was not related to absolute change in FEV_1pp (r = 0.26, $p > 0.05$) in this preliminary analysis. Increases in ASL correlated with improvements in FEV_1pp after 6 months of treatment (r = 0.58, $p < 0.05$) Stratification based on prior modulator status is in progress.

Conclusions: Results from the PROMISE HNE substudy demonstrate that HNECs derived from participants in a multicenter study reproduced improvements in CFTR activity and ASL depth, including the treatment effects of ELX/TEZ/IVA CFTR modulator treatment. At interim analysis, changes in Isc correlated with sweat chloride, and ASL depth correlated with clinical response (FEV_1pp), consistent with relationships established in ivacaftor-treated HNECs in the GOAL study. These findings provide additional confidence that HNECs are an important in vitro biomarker of treatment response to CFTR-directed therapies, including individual-level responses.

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Longitudinal improvements in clinical and functional outcomes following initiation of elexacaftor/tezacaftor/ivacaftor in patients with cystic fibrosis

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Background: The advent of highly effective modulator therapies (HEMTs), including elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA), for treatment of cystic fibrosis (CF) has resulted in remarkable clinical improvement for modulator-naïve patients and for those who have been treated with prior modulator therapies. Intranasal micro-optical coherence tomography (μOCT) has detected functional abnormalities in the mucociliary apparatus of people with CF. The objective was to characterize the effects of ELX/TEZ/IVA on nasal mucociliary clearance by μOCT and monitor the clinical changes conferred as a way to understand the effects.

Methods: Of 26 individuals aged 12 and older with at least one F508del mutation recruited, 24 were enrolled and followed over three visits: baseline and 1 (visit 2) and 6 months (visit 4) after initiation of ELX/TEZ/IVA therapy; the COVID-19 pandemic affected visit windows. Intranasal μOCT imaging was conducted at baseline and visit 2 as previously described; additional imaging for 18 months (visit 5) is in progress. Clinical outcomes, including percentage predicted forced expiratory volume in 1 second (FEV_1pp) and sweat chloride levels were computed as part of the parent Prospective Study to Evaluate Biological and Clinical Effects of Significantly Corrected CFTR Function (PROMISE study). A blinded investigator team analyzed in vivo μOCT parameters including mucociliary transport (MCT) rate, ciliary beat frequency (CBF), and periciliary liquid depth (PCL) after devising an improved stabilization algorithm. Analysis of airway surface liquid (ASL) depths was excluded because of the limited number of cases in which the necessary condition for measurement, which is preservation of a clear air layer between the mucus layer and the probe, was satisfied.

Results: Twenty-three subjects completed visits 1 and 2, and 18 completed visits 1, 2, and 4. Average age at baseline was 27 ± 8.7, 69% were female, and 43% were on prior two-drug modulator therapy. No significant change in body mass index was found between the visits. FEV_1pp increased significantly (10.9%, 95% CI, 76.1–98.4%) by visit 2 and persisted at visit 4 (10.6%, 95% CI, 87.7–107.0; $p < 0.001$). Sweat chloride levels decreased significantly at visit 2 (−36.6 mmol/L, 95% CI, 40.9–54.9 mmol/L) and visit 4 (−41.3 mmol/L, 95% CI, 34.9–51.8 mmol/L) at visit 4 ($p < 0.001$). Analysis of μOCT images revealed significant improvement in MCT rate (2.8 ± 1.5 mm/min at baseline vs 4.0 ± 1.5 mm/min at visit 2, $p = 0.048$), although no discernable changes were noted in CBF or PCL. When stratified based on use of prior modulator therapy, no significant differences were found for any μOCT metric. No significant correlations between change in MCT rates and change in FEV_1pp or sweat chloride from baseline to visit 2 were found.

Conclusions: Treatment with ELX/TEZ/IVA in people with CF, including those that were treatment naïve and those on prior modulator therapy, resulted in significant, sustained improvement in lung function and decreases in sweat chloride levels at ∼10 months, consistent with recently published reports. Functional improvements in MCT rate were evident after initiation of ELX/TEZ/IVA therapy, which may partially explain the findings of better whole-lung mucus clearance and reduction in chronic infections reported previously. μOCT imaging in people with CF is sensitive to the treatment effect of HEMT and suggests better mucociliary transport as a mechanism of action underlying the clinical benefits for lung health.

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Multicenter validation of the CF-ABLE score as a predictor of outcome and therapeutic response in cystic fibrosis

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Background: The CF-ABLE (age, body mass index (BMI), lung function, number of exacerbations in the last 3 months) score is an easy-to-use prognostic scoring system that has been validated in a large cohort from the national Cystic Fibrosis Registry of Ireland. This weighted score ranges from 0 to 7 and predicts risk of poor outcome, defined as death or requirement for lung transplantation, over time using commonly available clinical parameters such as age, BMI, forced expiratory volume in 1 second (FEV_1), and exacerbation frequency. In the original study describing the score, people with CF (PwCF) with a score of 5 or greater were deemed to have a 26% risk of poor outcome within 4 years. The present study aimed to evaluate real-world clinical performance of the CF-ABLE score as a clinical predictor in CF. Because clinical endpoints such as mortality and transplantation are typically difficult to meet in time-limited clinical trials, we also investigated the effect of double- and triple-combination CFTR modulator therapy on CF-ABLE score.

Methods: PwCF (n = 611) were recruited from three large specialist CF centers in the United States and Ireland. Participants were enrolled between 2013 and 2015 and followed clinically for 4 years from the point of enrollment unless they transitioned to a CF-ABLE score of 5 or greater within 2 years, in which case the 4-year follow-up period commenced at the time the score of 5 or greater was reached. In a parallel cross-sectional analysis of 60 PwCF chosen at random from the total cohort, the relationship between sputum inflammatory mediators associated with disease progression in CF and CF-ABLE score was also evaluated. Changes in CF-ABLE scores over time in PwCF who commenced ivacaftor, double-combination therapy (DCT) with lumacaftor/ivacaftor or tezacaftor/