Characterization of Bronchodilator Response in Children With Bronchiolitis Using Phenotypic and Genotypic Features

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4. RESEARCH STRATEGY

4.1. Significance.

4.1.a. Bronchiolitis Heterogeneity and the Need for Individualized Treatment. Bronchiolitis is a leading cause of pediatric morbidity and healthcare costs among children less than 2 years of age.[15–20] Bronchodilator treatments with beta-agonists, such as albuterol, are commonly used in conditions like asthma. Their efficacy in bronchiolitis remains controversial, yet use is common practice due to the heterogeneity in patient response.[21–35] Published evidence suggests that bronchodilators do not improve outcomes in all children with bronchiolitis;[22,33,34] however, meta-analyses can obscure the heterogeneity of treatment effects.[36] While *no prior studies have evaluated the genetics of bronchodilator response in bronchiolitis*, variations in bronchodilator effectiveness for asthma often depend on genomic factors.[49,50] Furthermore, studies have identified bronchiolitis phenotypes based on a child's clinical presentation,[37,56,57] environmental factors,[4,38–40] and molecular pathobiology,[37,41,45] suggesting the existence of treatment response heterogeneity, and supporting the identification of specific phenotypes that may guide the selection of bronchodilator treatment in children with bronchiolitis.[37,41,45]

Hence, the association between bronchiolitis phenotypes and their heterogeneous treatment response merits further investigation. Clinical features associated with a potential bronchodilator-responsive bronchiolitis phenotype include older age,[34,43,58–60] wheezing at presentation,[44,57] atopic conditions like atopic dermatitis or eczema,[38,39] and asthma in a first-degree relative.[44,61,56] The effective management of children with bronchiolitis is critical. Yet, bronchodilator administration is based on non-specific examination findings like wheezing or retractions with past medical and family history.[12] Importantly, our preliminary findings show these clinical evaluations are not reliably associated with a bronchodilator response. The heterogeneity of bronchodilator response in bronchiolitis highlights the complexity of this condition and underscores the need for individualized patient assessment and treatment decisions. This work is clinically meaningful because it will provide a targeted, rational approach to bronchodilator use by identifying a bronchodilator-responsive phenotype.

An evidence-based tool is needed to identify bronchodilator-responsive children with bronchiolitis because current practice leads to inefficient clinical decisions,[46] provider variation,[28] unnecessary treatments,[21] and increased resource use.[18]

4.1.b. Scoring Tools in Bronchiolitis. The American Academy of Pediatrics (AAP) recognizes the need for more research on scoring tools to set clinical standards and assess bronchiolitis severity and management.[21] Bronchiolitis scores have been developed to assist in decisions to initiate respiratory support.[62,63] However, *no current studies define airway response to treatment in children with bronchiolitis (Aim 1), differentiate statistically distinct from clinically significant respiratory changes (Aim 1), or determine the association between genetic and clinical variables on treatment response (Aim 2a/2b).*

4.1.c. Bronchodilator Use in Bronchiolitis. The use of bronchodilator therapy in the emergency department (ED) and hospital setting for bronchiolitis is not associated with reduced frequency of hospital admission, intensive care unit admission, return ED visits after ED discharge, invasive ventilation, or noninvasive ventilation.[33] However, bronchodilators remain widely used to treat bronchiolitis in the ED.[17–25] Bronchiolitis treatment has a diverse response pattern, partly due to short-term clinical improvement,[22] perceived responsiveness,[22] and the recognition of bronchiolitis as a heterogeneous disease.[64] This suggests that tailored bronchodilator therapies may offer advantages based on specific bronchiolitis phenotypes.[37,41,45,58] Establishing short-term responsiveness in children with bronchiolitis is challenging because no single treatment intervention reliably changes respiratory status in bronchiolitis,[21,33] unlike bronchodilator use in children with asthma. Provider-driven practice patterns lack an objective measure of a positive response to bronchodilator administration in bronchiolitis, and we will address this gap in the proposed research plan.

4.1.d. Genetic Associations with Bronchodilator Response. *No studies have evaluated the genetics of bronchodilator response in bronchiolitis.* However, genetic variants are associated with bronchodilator response and clinical outcomes in childhood asthma,[51–54,65–67] suggesting a bronchodilator-responsive genotype. Investigators who implemented genotype-directed prescribing of bronchodilators in children with asthma found that patients had a better quality of life and improved patient outcomes.[55] The identification of genetic variants or single nucleotide polymorphisms (SNPs) through Genome-Wide Association Studies (GWAS) allows for a targeted treatment approach in asthma, and this proposal will extend this paradigm to bronchiolitis (*Aims 2a*). Our proposed research will combine clinical characteristics with GWAS to characterize bronchiolitis phenotypes with bronchodilator-responsive genotypes. *Using GWAS data integrated with clinical characteristics to aid clinicians in real-time decision-making for bronchiolitis treatment is novel. This study will stratify patient information, provide reliable measures of disease severity, and predict bronchodilator response outcomes founded on patient-specific genetic information in children with bronchiolitis (Aim 2b).*

Based on my prior research, **we hypothesize that children with bronchiolitis who exhibit atopic history**

and specific physical findings (e.g., retractions, wheezing) have SNPs linked to bronchodilator response.

4.2. Innovation. The proposed research is innovative, representing the first effort in bronchiolitis research to: (1) define a minimum clinically important difference for bronchodilator response using the validated modified Tal score (MTS) in children with bronchiolitis (*Aim 1*); (2) explore associations between candidate SNPs and bronchodilator response to uncover genetic predictors in bronchiolitis (*Aim 2a*); and (3) develop a predictive model that integrates changes in the MTS with phenotypic and genotypic characteristics (*Aim 2b*). Data from Aims 1 and 2a will inform but not depend on Aim 2b. If Aim 2a identifies no SNPs, Aim 2b will still build a clinically relevant predictive model using Aim 1 data. By addressing these critical gaps, this study establishes the foundation for identifying predictive characteristics and creating a clinical prediction rule to enable rapid, evidence-based decision-making in the ED. These actions represent a transformative step towards precision treatment for children with bronchiolitis, offering a first-in-field approach to improving outcomes in pediatric bronchiolitis care.

Table 3. Potential Predictors of Bronchodilator-Response in Children with Bronchiolitis.

Demographic Factors	Historical Factors	Physical Examination Factors
Age Sex Race/Ethnicity	History of prematurity Immunizations (e.g., maternal RSV vaccination, infant RSV vaccination, monoclonal antibody) History of corticosteroid use Past medical history Family history Environmental exposure Pulmonary history	Weight-for-age General appearance Age-specific vital signs Peripheral blood oxygen saturation (SpO2) Work of breathing (e.g., retractions, grunting) Auscultatory findings (e.g., wheezes, decreased or uneven breath sounds) Impression of disease

4.3. Preliminary Results. During the PI's K12, we conducted a double-blind, randomized, placebo-controlled trial of a single albuterol dose in children (3 to 24 months) with bronchiolitis to identify factors associated with bronchodilator response. Preliminary data from 82 participants (July 2022 to December 2023) showed that ≥25% of children treated with albuterol experienced at least a 2-point reduction in symptom severity by MTS, indicating a high responder group. However, historical and physical findings (before or after treatment) (**Table 3**) could not reliably predict bronchodilator response. GWAS analysis revealed candidate SNPs (**Table 4**) associated with bronchodilator response, including coding and non-coding SNPs, highlighting the need for further investigation into the genetic architecture of bronchodilator response. We will expand enrollment and biospecimen using the PI's K12 study infrastructure **(Section 4.4.1.h.)**.

Table 4. Candidate SNPs known to be associated with bronchodilator response.

Chr	SNP	Gene	Function	MAF
8	rs6988229	COL22A1	Intronic	0.204
7	rs73294475	CRHR2	Intronic	0.056
1	rs10746419	PLXNA2 / LAMB3	Intergenic	0.531
22	rs518350	MIAT / MN1	Intergenic	0.185
20	rs16995064	PLCB1	Intronic	0.08
7	rs1419555	POT1 / ENSG00000197462 / GRM8	Intergenic	0.383
6	rs13437006	GRIK2 / HACE1	Intergenic	0.228
6	rs2781659	AKAP7 / ARG1	Intergenic	0.444
4	rs17701271	IL15 / INPP4B	Intergenic	0.179
5	rs17834628	CTNND2 / LINC02220 / DNAH5	Intronic	0.321
5	rs1017451	CTNND2 / LINC02220 / DNAH6	Intronic	0.302
6	rs13200833	TINAG / FAM83B	Intergenic	0.383
3	rs892940	THRB-AS1	Intronic	0.673
5	rs1042714	ADRB2	Missense	0.704
5	rs17495520	TENM2	Intronic	0.142
9	rs10511905	/ ACO1	Intergenic	0.179
22	rs6002674	TCF20 / NFAM1	Intergenic	0.185
2	rs295114	SPATS2L	Intronic	0.407
5	rs1042713	ADRB2	Missense	0.34
12	rs66544720	*PLEKHA5 / PDE3A*	Intergenic	0.346

SNP = single nucleotide polymorphism, Chr = Chromosome, MAF = mean allele frequency.

4.4. Approach. *This proposal aims to characterize phenotypic and genotypic variations of children with bronchiolitis and their association with bronchodilator response.*

4.4.1. Specific Aim 1. *Define airway responsiveness to bronchodilator treatment in children with bronchiolitis using the change in respiratory score.*

4.4.1.a. Rationale for modified Tal Score (MTS). There is no standard definition of airway response nor consensus on the best respiratory scoring tool for assessing treatment response in bronchiolitis.[68–70] The validated MTS is the most appropriate clinical scoring tool[71–78] for this proposal based on clinical applicability, ease of use,[72] practicality, and high inter-rater agreement,[79] even among physicians with varying experience and training levels[71,80] (**Table 5**). The MTS includes respiratory parameters documented as part of the ED standard of care. The MTS score ranges from 0 to 12 points, with higher scores indicating greater respiratory distress. A limitation in all respiratory scoring tools, including the MTS, is that there is no well-established correlation between statistically significant changes in respiratory scores and clinically meaningful differences. We will identify the clinically relevant change in bronchodilator response by determining the minimum clinically important difference (MCID) in the MTS. Identifying the MCID helps standardize the evaluation of treatment efficacy, ensuring interventions are guided by clinically meaningful outcomes rather than statistically significant but subclinical changes. The MCID represents the smallest change in a clinical (respiratory) measurement that parents/caregivers or clinicians perceive as beneficial, meaningfully influencing clinical decision-making or having a tangible impact on the patient's symptoms.

Table 5. Modified Tal Score.

Variable	0 points	1 point	2 points	3 points
Respiratory rate (breaths/min)				
Age <6 months	≤40	41-55	56-70	≥71
Age ≥6 months	≤30	31-45	46-60	≥61
Age ≥12 months	≤20	21-35	36-50	≥51
Wheezing/ Crackles	None	Expiration only	Expiration and inspiration with stethoscope only	Expiration and inspiration without a stethoscope
O_2 Saturation (room air)	≥95	92-94	90-91	≤89
Accessory respiratory muscle utilization	None (no chest in-drawing)	+ Presence of mild intercostal in-drawing	++ Moderate amount of intercostal in-drawing	+++ Moderate or marked intercostal in-drawing, with presence of head bobbing or tracheal tug

4.4.1.b. Hypothesis. Bronchodilator response can be defined by establishing a threshold for airway response

using the validated MTS. *To accomplish this aim,* we will establish the MCID and apply it to the validated MTS using anchor-based (subjective) and distribution-based (objective) methods.[81] We will use this result to identify high and low responders to bronchodilator therapy. Our preliminary data suggest that ≥25% of children with bronchiolitis will be classified as high responders to bronchodilators using a dichotomous outcome. The MCID responses will identify characteristics that indicate which patients will benefit from bronchodilator therapy.

4.4.1.c. Study Design. We will conduct a prospective, double-blind, randomized, placebo-controlled trial (RCT) of a single albuterol dose in children ages 3 to 24 months who present with bronchiolitis to the Nemours Children's Health-Florida (NCH-FL) ED. Albuterol is a bronchodilator commonly used to treat children with bronchiolitis and asthma.[82] This study will identify predictors of bronchodilator responsiveness among children with bronchiolitis. An RCT provides the best design for an unbiased collection of patient information and respiratory assessment outcomes. We will use the infrastructure from the previous K12 led by the PI to collect clinical data and biospecimens **(Section 4.3).**

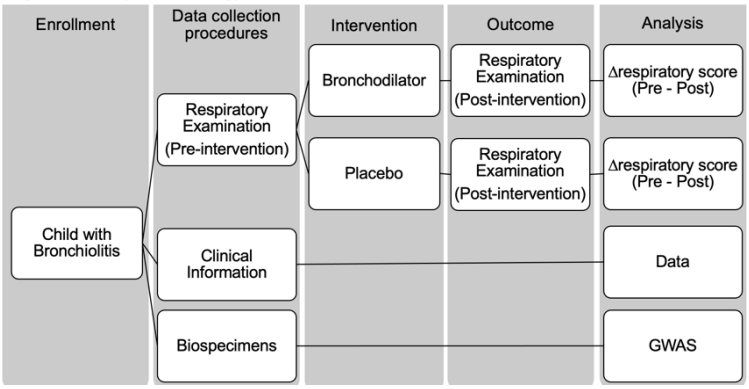
4.4.1.d. Study Population. Eligible patients will include infants (3 to 24 months) with bronchiolitis. Bronchiolitis is a clinical syndrome involving lower respiratory tract symptoms[21] as identified by the treating provider(s). We will include children who either have no history of prematurity or have a history of prematurity but without associated co-morbidities (see exclusion criteria). We will exclude patients previously enrolled in the PI's K12 study, patients with a documented history of asthma or reactive airway disease, co-morbidities affecting airway response (e.g., chronic lung disease, bronchopulmonary dysplasia, bronchiectasis, congenital heart disease, immunodeficiency, neurologic condition), and diagnosis of pneumonia by chest radiography. Other exclusion criteria include inhaled, nebulized, or oral *corticosteroid* use within 72 hours of ED evaluation and inhaled, nebulized, or oral *bronchodilator* administration within 4 hours of ED arrival.

Table 6. Data Collection Procedures

Case Report Forms
Parent – demographics, history of prematurity, immunizations, past medical history, pulmonary history, family history, environmental exposure
Physician – examination findings, disposition, impression of disease severity
Specimens Collected
Blood
Urine
DNA buccal swab
Nasopharyngeal swab
Data from Electronic Medical Record
Vital signs
ED resource use – laboratories, radiographs
ED escalation of care – oxygen support
ED return visit
Respiratory Examination Performed upon Enrollment
Pre-intervention
Post-intervention

4.4.1.e. Patient Recruitment, Data Collection, and Management. Enrollment will not delay clinical management. After identifying each potential participant, a research staff member (RSM) will confirm eligibility, obtain informed consent, and initiate data collection procedures (**Table 6**). Data variables are summarized in **Table 3**. A clinically trained RSM will perform an examination and compute the MTS (pre-intervention score). Twenty to 30 minutes after nebulization of albuterol or placebo is complete, the patient will be re-evaluated, and the MTS will be computed (post-intervention score). The MTS delta (Δ) respiratory score is the numerical change observed between the pre-intervention and the post-intervention score. The parent/caregiver and treating provider will be asked the global transition question (GTQ):[83] "Since treatment, has there been any change in the child's respiratory function?." DNA buccal swab specimens (for genotype characterization), and blood, urine, and nasopharyngeal swabs (for future epigenetic studies) will be collected. Data collected for this study, including the results from the MTS Δrespiratory score, will not be shared with the treating provider or used for medical decision-making. The treating provider will direct continued patient care after treatment with the blinded intervention drug, and study participation will be concluded. The expected duration for the study participation is 30-60 minutes. As in the K12 workflow, enrollment will be performed by a research staff member on weekdays from 7a-11p. The PI will be on-call for overnight and weekend enrollment.

Figure 1. Study methodology.



4.4.1.f. Sample Strategy, Randomization, and Rationale for Saline Placebo. Patients will be randomized to receive either a bronchodilator (albuterol sulfate 2.5mg, 3mL inhalation solution) or normal saline (NS) as a placebo (sodium chloride 0.9%, 3mL inhalation solution) (**Figure 1**). Nebulized NS helps thin mucus and aids in airway clearance.[21,84] It acts as a placebo, replicating the NS component of the albuterol sulfate in the intervention group.[82] NS and albuterol are commonly used in ED settings for bronchiolitis treatment. The parent/caregiver, treating provider, and RSM will be blinded to intervention allocation. The bedside nurse will be unblinded to ensure clinical safety.

4.4.1.g. Specimen Handling and Storage. Within 2 hours of collection, urine, and serum prepared from whole blood are aliquoted and stored frozen at -80°C until subsequent analysis. The nasopharyngeal swabs are placed

in a viral transport medium, RNA-seq Lysis Buffer, and MagMAX Lysis Buffer. After processing, samples are frozen at -80°C in the NCH Clinical Research Center (CRC) and stored until ready for analysis. The buccal swab for DNA (Oragene OGD-575) (DNA Genotek, Inc., Stittsville, Canada) will remain stored in nucleic acid stabilization media at room temperature until subsequent analysis. The PI actively participates in biospecimen collection and prepares samples for future epigenetic studies.

4.4.1.h. Current and Projected Enrollment. In the PI's K12 study, we enrolled 126 children with bronchiolitis (July 2022 through January 2025) and collected over 300 biospecimens (buccal DNA, nasopharynx, serum, and urine). In the proposed study, we are expanding patient enrollment, data collection, and biobanking of specimens. Based on accrual projections accounting for seasonality and bronchiolitis prevalence, we anticipate a cumulative enrollment of 400 children by the end of Q4 in Year 4 of the K23 award (**Table 7**).

Table 7. Monthly accrual projection, accounting for seasonality and bronchiolitis prevalence.

Enrollment period	1	2	3	4	5	6	7	8	9	10	11	12	Cumulative patients enrolled
2022							8	0	14	2	6	3	33
2023	2	4	2	0	0	0	1	3	0	7	11	18	81
2024	0	1	1	6	3	3	2	3	4	9	8	2	123
2025	6	4	3	3	3	3	3	3	6	7	8	8	180
2026 (K23 Year 1)	4	4	3	3	3	3	3	3	6	7	8	8	235
2027 (K23 Year 2)	4	4	3	3	3	3	3	3	6	7	8	8	290
2028 (K23 Year 3)	4	4	3	3	3	3	3	3	6	7	8	8	345
2029 (K23 Year 4)	4	4	3	3	3	3	3	3	6	7	8	8	400

Shading indicates patient enrollment to date.

4.4.1.i. Methods.

Outcome Measures. The overall goal is to identify the MCID in the MTS in children with bronchiolitis from parents/caregivers and treating providers' perceived improvement in respiratory response, which will be used in conjunction with patient characteristics to identify children with the likelihood of experiencing the greatest benefit from bronchodilator therapy. The **primary outcome** is to establish the MCID in the MTS using anchor-based (subjective) and distribution-based (objective) methods to identify high and low responders to bronchodilator therapy. **Secondary outcomes** will be evaluated in 2 ways: (1) We will determine the optimal cutoff value in the MTS to differentiate high versus low bronchodilator responsiveness, and (2) We will compare the ∆respiratory score for high versus low responses to determine a statistically significant response.

Statistical Analysis. The MCID[85] will be calculated within our cohort using anchor-based and distribution-based methods[81] (two methods each). The anchor-based MCID will determine the MTS ∆respiratory score associated with the caregivers' and treating providers' perceived improvement in respiratory response, as assessed by the GTQ.[83] An anchor establishes if the patient is better after treatment as a dichotomous variable (yes/no) and as a ranked continuous variable on a 15-point scale (-7 to +7, very much worse to very much improved), as assessed by responses from the caregiver(s) and the treating provider(s). A response of +1 to +2 will be interpreted as a "small" perceived improvement in respiratory function. The MCID will be calculated from the mean MTS ∆respiratory score for caregivers reporting a "small" perceived change in the GTQ. A receiver operator curve (ROC)[86] will be constructed from the dichotomous outcome of the GTQ as a function of the MTS ∆respiratory score, and the Youden index from the area under the curve will determine the optimal MCID.[87]

The distribution-based method will calculate the MCID as 0.5x the standard deviation (SD)[88] of the MTS ∆respiratory score mean and one standard error of the mean[89] of the MTS ∆respiratory score. The effect size will be expressed as the standardized mean difference and obtained by dividing the difference between the means of the pre- and post-intervention scores by the SD of the pre-intervention score.[89]

4.4.1.j. Aim 1: Potential Problems and Alternative Approaches.

Seasonal Variation of Bronchiolitis. Florida's subtropical climate supports the year-round circulation of respiratory viruses that cause bronchiolitis (e.g., respiratory syncytial virus (RSV) and influenza), with a peak from November to March.[90,91] Bronchiolitis ED visits at NCH-FL have remained steady (>600 cases annually) over the last 2 years. Monthly accrual projections are adjusted for seasonality and bronchiolitis prevalence to guide enrollment.

Severity of Bronchiolitis. Bronchiolitis severity may be self-selected, with milder cases seen in primary care and moderate-to-severe cases in the ED. We will stratify pre-treatment scores by severity and incorporate severity into our predictive modeling, ensuring a comprehensive illness analysis.

Immunization Against Bronchiolitis-Causing Viruses. The 2023-2024 national immunization rate of children for COVID-19 is 14% (95% CI 13-15%),[92] influenza is 52% (95% CI 51-53%),[92] and RSV is 41%.[93] Collected variables will include maternal and infant RSV vaccination and monoclonal antibody receipt. In 2024, 476/912 (~52%) bronchiolitis cases at NCH-FL were non-RSV, highlighting the prevalence of other viral causes and underscoring that RSV preventive strategies are unlikely to impact patient enrollment substantively.

Role of Age and Bronchodilator Responsiveness. Age may play a role in disease severity and bronchodilator response. Rather than exclude children of certain ages, we will account for age in statistical modeling, and we hypothesize that age will be either an effect modifier or a potential predictor of bronchodilator response.

Selection Bias. Since the original submission, an additional RSM was included to expand enrollment hours (weekdays, 7a-11p). The PI will be on-call for overnight and weekend enrollment. RSMs will identify missed eligible children with bronchiolitis who visited the ED and collect their historical and clinical findings from the

electronic health record. We will compare predictors and outcomes for enrolled participants versus missed participants versus candidate subjects whose caregivers declined participation to assess for selection bias.
Sampling Bias. We will use ethnically and racially diverse research coordinators and assistants to encourage enrollment of children of all races and ethnicities. Patient enrollment and data collection will be performed in English or Spanish based on parent/caregiver preference, ensuring linguistic accessibility and cultural relevance.
Missing Data. Missing variables will be verified through medical chart review and communication with the family as needed. Multiple imputation and sensitivity analyses will be performed to quantify the effect of the missing outcome data and assess the robustness of the study findings.
Drug Shortage. Despite a national shortage of albuterol, the PI's K12 maintained adequate supplies of albuterol.

4.4.2. *Specific Aim 2. Identify the associations between candidate genetic variants and bronchodilator response among children with bronchiolitis.*

4.4.2.a. Rationale for Genome-Wide Association Studies (GWAS). No genetic studies have evaluated bronchodilator response in bronchiolitis. Several genetic studies have explored children's predisposition to developing bronchiolitis[94–96] and its relationship with the later development of childhood asthma.[59,97–102] Studies of childhood asthma have shown that genes associated with bronchodilator response are familial,[103,104] suggesting that a significant component of the response to beta-adrenergic agonists (bronchodilators) is heritable.[54,105] The proposed GWAS will assess known SNPs (such as in ADRB2) and explore additional genetic factors, including those beyond beta-adrenergic pathways, to inform the development of a clinically applicable predictive algorithm based on genotype-phenotype correlations. We hypothesize that, as in asthma, genetic predictors associated with bronchodilator response in bronchiolitis are associated with specific patient characteristics identified as clinical, historical, and physical examination factors (**Tables 3-6**).
4.4.2.b. Hypothesis. SNP alleles will be associated with a bronchodilator-responsive phenotype in bronchiolitis.
4.4.2.c. Methods.
Outcome Measures. The **primary outcome** is to identify candidate SNPs, rank-ordered by the strength of their association with the MTS ∆respiratory score based on an odds ratio (dichotomous response) or a beta coefficient (continuous response) and filtered by adjusted p-value. A **secondary outcome** will be the identification of candidate SNPs and their association with the disaggregated components of the pre- and post-intervention MTS.
Processing and Analysis of Genetic Samples. Genomic DNA will be purified from saliva collected in Oragene OGD-575[106] and quantitated using a fluorescent dye-binding assay[107] (Quant-iT PicoGreen dsDNA Assay, Thermo Fisher Scientific, Waltham, MA). DNA samples will be diluted to 10 ng/μL in deionized, sterile water, and 20 μL are aliquoted into a 2.2 mL Eppendorf DeepWell 96-well plate (Eppendorf Biotech, Hamburg, Germany) and sealed with an adhesive-backed, foil microplate sealing film. Analyses will contain 5% duplicate samples, two positive controls, and a designated blank to assess reproducibility and background contamination. The Gene Expression & Genotyping group of the University of Florida's Interdisciplinary Center for Biotechnology Research (UF-ICBR) will genotype the specimens using the Precision Medicine Diversity Array[108] on the Applied Biosystems Gene Titan platform (Thermo Fisher, Waltham, MA). The instrument's Axiom Analysis Suite software[109] assesses overall array quality from the Dish Quality Control (DQC). DQC is based on quantile normalized intensities of probe sequences for non-polymorphic genome locations and is a measure of signal-to-noise ratio for specific hybridization of both A/T and G/C probe sets for each of the 96 arrays on the plate. Samples with a DQC value less than the default DQC threshold are excluded. Additional filtering is performed based on the sample call rate threshold (typically ≥97%). Plates with an average quality control (QC) call rate of passing samples <98.5% (>1 failing sample on a 96-well plate) are considered non-passing plates. The average QC call rate of passing samples on the plate represents the mean of the QC call rates of samples passing DQC and the 97% QC call rate thresholds. We anticipate an average QC call rate of ≥93% (saliva specimen). The best-performing probe set for each SNP will be identified based on 17 SNP-level QC metrics. The software reports SNP genotype calls, insertions/deletions (indels), multi-allelic variants, and copy number variants, and data is exported in variant call format. We anticipate a concordance between duplicate samples >99.8%. Additional genotype information will be imputed using published methods.[110] Imputation accuracy will be assessed by comparing the discordance rate (% of discordance between imputed and masked genotypes) with the missing rate (% of no calls made for masked genotypes) on different imputation genotype posterior probabilities[111] thresholds. Sensitivity and false positive rates will be calculated. We expect the imputation accuracy (mean R^2) to be >0.9 for SNPs with minor allele frequency (MAF) >0.05.
Covariates. Demographics (i.e., age, sex, race, ethnicity), ED vital sign abnormalities (i.e., tachycardia, tachypnea, hypoxia), intervention drug, disease severity, and ED physical examination findings.
Statistical Analysis. *Genetic Predictors of Bronchodilator Response in Bronchiolitis.* Variants associated with MTS ∆respiratory scores will be identified using multivariate linear regression models in PLINK 2.0.[112,113] The PLINK 2.0 PHENO and COV files will be built by combining outcomes data (including MTS ∆respiratory scores),

other covariates, and the first 5 dimensions of the principal components analysis (PCA). The initial QC steps will be conducted in PLINK 2.0[114,115] and include; (1) the removal of individuals with ≥10 % SNP missingness and SNPs with <95% call rate; (2) SNPs with a MAF <5% (very low power); (3) SNPs with deviations from Hardy–Weinberg equilibrium in the control group (potential genotyping issues); (4) SNPs with a difference in heterozygosity between sexes >7% or absolute difference in call rate between sexes >2.5%; (5) linkage disequilibrium (LD) pruning (100Kb window, r2 threshold 0.8) to keep one SNP with the highest MAF that is a representative of a cluster of SNPs in LD; (6) relatedness; and (7) correcting for population stratification by PCA.

The analysis will include SNPs associated with bronchodilator response in children with asthma.[52,53,116] The association between the MTS ∆respiratory scores and SNPs coded using an additive model (i.e., the effect of each additional minor allele) will be evaluated to perform a linear regression analysis for each SNP. Regression coefficients and 95% confidence intervals will determine the difference in mean treatment response (bronchodilator versus placebo) corresponding to each additional minor allele. Data transformations of MTS ∆respiratory scores will be considered to address distributional skewness. A Bonferroni correction for multiple testing will be utilized.[117] All statistical tests will be two-sided.

4.5. Sample Size. We will conduct a sub-analysis of all SNPs interrogated genome-wide. Candidate SNP tests will be restricted to ≤20 (Bonferroni correction ≥0.0025) of the top replicated candidate SNPs identified from previous studies.[51–54,66,116,118,119] Assuming an additive genetic model, a sample size of 200 patients with bronchiolitis in each randomly assigned group (total cohort N= 400) and a Cohen's d effect size of 0.8 for bronchodilator response in SNP carriers relative to noncarriers and a response rate of 0.25, with an alpha of 0.0025, we will have 80% power at a MAF of 7.6% to significantly associate bronchodilator response and SNP(s).

4.7. Research Timeline. **Table 8** outlines the anticipated timeline for the 5-year research plan.

4.8. Future Directions. Data generated from this study will inform a future NIH R01 grant, for which I will apply during Year 5 of the K23 award, focused on the derivation and external validation of an evidence-based prediction rule. We will also have developed a large biorepository in children with bronchiolitis to facilitate the future discovery of additional candidate SNPs and biomarkers for bronchodilator response.

Table 8. Timeline of Proposed Research Activities.

	Pre-Grant	Y1	Y2	Y3	Y4	Y5
IRB Approval						
Enrollment (n)						
Aim 1 Data Analysis						
Manuscript Writing Aim 1						
Aim SNP Profiling/Analysis						
Data Cleaning						
Aim Data Analysis						
Manuscript Writing						
Prepare NIH R01 Grant Application						
NIH R01 Grant Submission						