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CFTR gene variants, air pollution, and childhood asthma in a California Medicaid population

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Abstract

Carriers of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene ("carriers") have been found to have an increased risk of persistent asthma. However, it is unclear at what level of CFTR function this risk exists and whether it is modified by asthmogens, such as air pollution. We conducted a retrospective cohort study of children born in California between July 2007 and December 2013, linking *CFTR* genotype data from the California newborn screening program to Medicaid claims records through March 17, 2020 to identify asthma cases, and to air pollution data from CalEnviroScreen 3.0 to identify levels of particulate matter with diameter 2.5 microns or smaller ($PM_{2.5}$). Log-binomial regression models for asthma risk were fitted, adjusting for race/ethnicity and sex. Compared to population controls, carriers had higher risk of asthma (adjusted risk ratio (aRR) = 1.29, 95% confidence interval (CI): 0.98, 1.69; $p < 0.1$). Other non-CF-causing variants on the second allele did not appear to further increase risk. Genotypes with the greatest asthma risk were F508del with an intron 10 T7 or (TG)11T5 in *trans* (aRR=1.52, 95% CI: 1.10, 2.12). This association was higher among children living in areas at or above (aRR = 1.80) versus below (aRR = 1.37) the current national air quality standard for $PM_{2.5}$, though this difference was not statistically significant ($p_{interaction} > 0.2$). These results suggest carriers with CFTR functional levels between 25% and 45% of wildtype are at increased risk of asthma. Knowledge of *CFTR* genotype in asthmatics may be important to open new CFTR-related treatment options for these patients.

KEYWORDS

child health, cystic fibrosis transmembrane conductance regulator, gene-environment interaction, neonatal screening

1 | INTRODUCTION

Pediatric asthma is a chronic inflammatory airway disorder with polygenic and environmental etiology,[1] with symptoms including shortness of breath, cough (including nocturnal cough), wheezing, and chest tightness. The cystic fibrosis transmembrane conductance regulator (*CFTR*) gene has been studied for over three decades as a possible cause of asthma. The resulting protein, CFTR, regulates chloride and bicarbonate transport across cell membranes in many organs, including the lungs. Dysfunctional variants on both copies of the gene cause cystic fibrosis (CF).[2] *CFTR* variants are common in the population, with 1 in 29 persons carrying a dysfunctional variant on a single allele (carriers).[3] In addition to having one known CF-causing *CFTR* variant, 29% of carriers identified by newborn screening in California have another variant not likely to cause CF on the second allele.[4] In such persons, sometimes diagnosed as newborns with CFTR-related metabolic syndrome,[5] a range of disorders such as male infertility, pancreatitis, and sinusitis has been found.[6]

In 2016, Nielsen published a meta-analysis of 15 studies that demonstrated the odds ratio (OR) for asthma in CF heterozygotes versus non-carriers was significantly elevated (OR = 1.61, 95% confidence interval (CI): 1.18–2.21).[7] Because these and other past studies[8] have used varying CF carrier definitions, most focusing on a limited panel of *CFTR* variants or polymorphisms, it remains uncertain whether increased asthma risk is associated with being (i) a true *CFTR* carrier (CF-causing variant on only one allele) with approximately 50% of CFTR wildtype function, or (ii) a supposed *CFTR* carrier, but actually having variants on both alleles, with overall CFTR function less than 50%. This distinction is critical in screening, referral, diagnostic, and treatment options for these persons.

Environmental factors such as air pollution are known to precipitate asthma exacerbations.[9] Associations between incident asthma and some of these factors have been identified using administrative datasets from Medicaid, a United States government program that provides health insurance coverage to low-income families.[10,11] Environmental factors such as tobacco smoke may also have a direct effect on lowering CFTR function at the cellular level.[12]

We linked *CFTR* genotype data from the California Department of Public Health (CDPH) Genetic Disease Screening Program (GDSP), which screens nearly all California newborns, with Medicaid records on asthma care from the California Department of Health Care Services, and air pollution data from the California Environmental Protection Agency CalEnviroScreen version 3.0 environmental health screening tool. Using this linked dataset, we addressed the following questions: (1) Is persistent childhood asthma associated with a single CF-causing variant? (2) Does having an additional non-CF-causing *CFTR* variant on the second allele further increase asthma risk? (3) Is persistent asthma associated with specific *CFTR* genotypes? (4) Does residential air pollution modify the effect of *CFTR* genotypes on asthma risk? (5) Is asthma control reduced in children with *CFTR* genotypes associated with increased asthma risk? We hypothesized that additional non-CF-causing variants increase asthma risk, air pollution exposure elevates this risk, and *CFTR* carriers have reduced asthma control.

2 | MATERIALS AND METHODS

2.1 | Study population

We conducted a retrospective cohort study of low-income children born in California. First, we used CF newborn screening (NBS) records to define the study population base. The CF NBS algorithm is described in detail elsewhere.[4] Briefly, immunoreactive trypsinogen (IRT) levels are measured in newborn bloodspots, and newborns with levels in the top 1.6% undergo genetic testing for a panel of forty common CF-causing variants ("panel variants"). Specimens with one panel variant undergo DNA sequencing of the *CFTR* gene, whereupon additional variants may be identified. We utilized CF NBS screening records for children born between the initiation of the screening program on July 16, 2007, through June 28, 2017, when cohort assembly for the study began, to create three study groups: (1) control group with IRT values below the genetic testing cutoff ("Low IRT controls"); (2) control group with IRT values at or above the genetic testing cutoff but with no panel variants identified ("High IRT controls"); and (3) a carrier group with one panel variant ("carriers"). Carriers are a combination of (i) children with only one panel variant whose parents are referred by NBS for telephone counseling, and (ii) children with one panel variant and other variants identified through *CFTR* sequencing who are referred to CF specialty centers for evaluation and diagnosis, many of whom receive an initial diagnosis of CFTR-related metabolic syndrome. Low IRT represents approximately 98.4% of California newborns and is generally less likely than High IRT to contain non-panel *CFTR* variants, given the association between elevated IRT and the likelihood of having *CFTR* variants.[13] However, because carrier identification only occurs within the High IRT group, including a High IRT control group may alleviate bias associated with other contributors to elevated IRT that are also associated with asthma, such as preterm birth.[14] Thus, we report comparisons with each referent group separately, as each has certain inferential advantages. From the NBS database, we randomly sampled 10,000 individuals from the Low IRT and High IRT populations and included all carriers, providing the statistical power (80%, $\alpha = 0.05$) to detect a risk ratio for asthma of 1.27 assuming that 50% of the population would link to Medicaid records and asthma prevalence would be 9.4%.[15]

This study population underwent multiple exclusions (Figure 1). First, CF converters known to the NBS program, children with a genotype consistent with CF according to Clinical and Functional Translation of *CFTR*[16] (www.cftr2.org) (CFTR2), and duplicate NBS records were excluded. The study population was subsequently linked to Medicaid eligibility and claims data by the child's first name, last name, and birth date, to ascertain asthma outcomes. NBS records between 2014 and 2017 lacked the child's first name, thus preventing Medicaid linkage and restricting the study population to

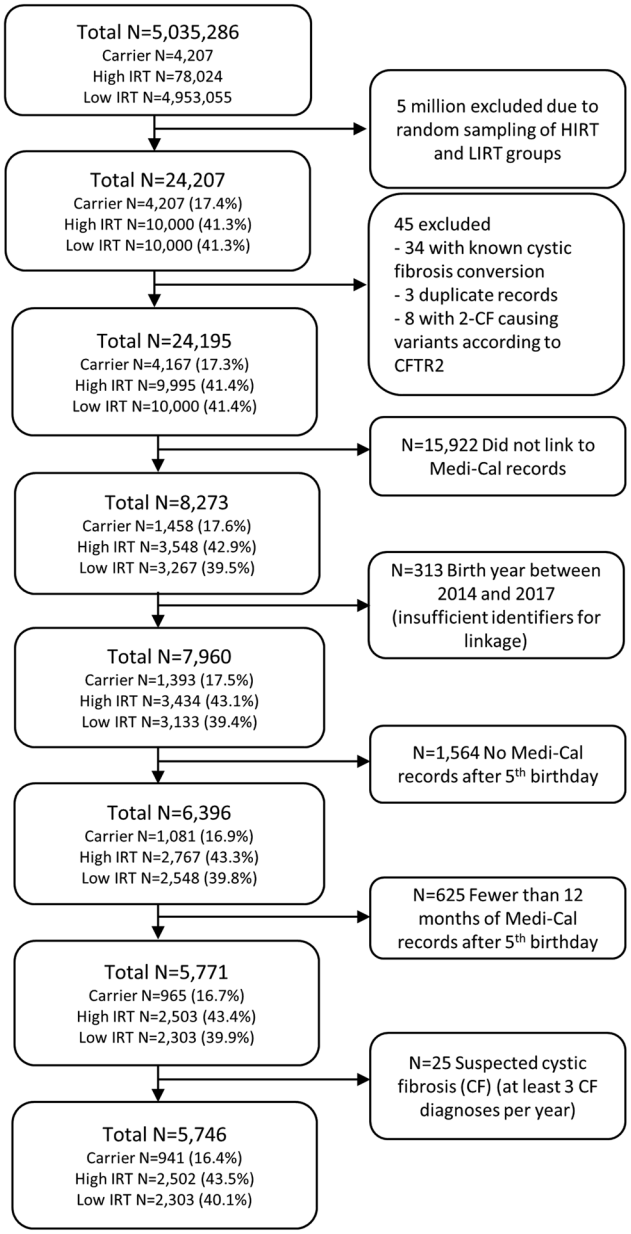

FIGURE 1 Flowchart detailing study population selection steps beginning with all newborns in the California newborn screening program born between July 16, 2007 and June 28, 2017. Percentages indicate the composition of the study population at each step by the three study groups of interest (*CFTR* carriers and the two referent groups, high IRT and low IRT). CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator gene; CFTR2, Clinical and Functional Translation of *CFTR*; HIRT, high immunoreactive trypsinogen; IRT, immunoreactive trypsinogen; LIRT, low immunoreactive trypsinogen

birth years from 2007 through 2013. Follow-up was truncated on March 16, 2020, to avoid the influence of changing trends in health services utilization caused by California's COVID-19 stay-at-home order. We excluded subjects who only had Medicaid claims information before the fifth birthday, since spirometry, the gold standard for asthma diagnosis, can be challenging to perform in younger patients.[17] Additionally, we required at least 12 months of follow-up to provide sufficient time for identification of asthma-related care. Finally, children with at least three primary diagnoses of CF (International Classification of Diseases 9th Revision Clinical Modification (ICD-9-CM) 277 or ICD-10-CM E84) per year of follow-up were removed on suspicion of CF conversion. This definition was chosen to approximate the consistent, repeated follow-up that children with CF typically receive.

2.2 | Carrier genotype classification schemes

We implemented several schemes in disaggregating the carrier group. We first distinguished individuals with a single panel variant from those with additional variants. Next, we classified genotypes according to CFTR2 variant categories and utilized parent genotype (available from 16% of carriers) and other available data to distinguish carriers with approximately 50% function (i.e., those with no variants in *trans*) from those with less than 50% function (i.e., those with varying clinical consequence, unknown significance, or non-CF-causing variants in *trans*). Upon discovering that additional variants in *trans* were not associated with higher asthma risk and after exploratory analyses of high-risk genotypes, we created a genotype subgroup composed of Intron 10 (TG)mTn Poly-variant tract haplotypes in *trans* known to confer reduced CFTR function,[18] specifically T7 (c.1210-12T[7]) or (TG)11T5 (c.1210-33_1210-6GT[11]T[4]) in *trans* with an F508del (c.1521_1523delCTT) variant, regardless of other variants in *cis* or *trans*, versus other carriers. We used percent wildtype function values for each allele from Sterrantino et al.[18] and McCague et al.,[19] summing them to derive total expected percent wildtype function values (e.g., F508del (1%) plus (TG)115T (24%) equals 25%).

2.3 | Air pollution

We utilized CalEnviroScreen (CES) version 3.0, an environmental health screening tool containing census tract-level pollution indicators, to estimate early life air pollution exposure. We selected particulate matter with a diameter under 2.5 microns ($PM_{2.5}$) as the measure of asthma-relevant air pollution, as these particles penetrate deep into the airways and are implicated in pediatric asthma development and exacerbations.[20] The methods used to construct the $PM_{2.5}$ indicator are described in detail elsewhere.[21] Briefly, concentrations were estimated at the census tract centroid from a kriging model utilizing air monitoring station measurements, or satellite data when stations were too far from the centroid, over a three-year period (2012–2014). For each child, the maternal address at the time of birth was extracted from NBS records and geocoded to the census tract. $PM_{2.5}$ concentrations were subsequently linked to the addresses to estimate prenatal or early childhood exposure, depending on the child's birth year. Approximately 5% of addresses could only be geocoded to the ZIP code or were unable to be

geocoded. When it was only possible to geocode to the ZIP code, we imputed the census tract concentration using the average concentration across all census tracts within the ZIP code.

2.4 | Outcome definitions

Asthma definitions were based on episodes of care (EOC), defined as unique service start dates in an individual's Medicaid claims record. We defined persistent asthma as the occurrence of at least two EOCs containing a primary diagnosis of asthma (ICD-9-CM 493 or ICD-10-CM J45) after the fifth birthday. To quantify asthma control, two measures were constructed: the average annual asthma exacerbation rate and average annual short-acting beta agonist (SABA) prescription fill rate.[22] Asthma exacerbations were defined as asthma-related unscheduled outpatient visits (one-day EOC containing an asthma primary diagnosis and at least one acute asthma treatment procedure code), emergency department visits (one- or two-day EOC containing an asthma primary diagnosis and an emergency room place-of-service code), or inpatient hospitalizations (EOC lasting two or more days containing an asthma primary diagnosis and inpatient claim code). We obtained National Drug Codes (NDCs) for all SABA medications listed in the American Academy of Allergy, Asthma, and Immunology asthma medications list and linked these to NDCs in the claims data to identify SABAs. Total counts of exacerbations or prescriptions were divided by follow-up years to obtain average annual rates.

2.5 | Statistical analyses

To assess the association between carrier status and asthma prevalence, we employed log-binomial models to derive estimates of the risk ratio (RR) and associated 95% confidence interval (CI). In adjusted models, we included two *a priori* selected confounders: race/ethnicity (non-Hispanic White; non-Hispanic Black; Hispanic; Asian, Native American, multiracial, or other (non-Hispanic Asian/other)) and sex (male or female), both extracted from NBS records. Unknown race/ethnicity was imputed using the most frequently listed race/ethnicity in Medicaid eligibility records. Models were fit with Low IRT controls as the referent group, and again with High IRT controls as the referent group. While we defined statistical significance at $p < 0.05$, we note estimates that approach significance with $p < 0.1$. For air pollution effect modification analyses, the CES 3.0 $PM_{2.5}$ indicator was dichotomized using the primary $PM_{2.5}$ National Ambient Air Quality Standard (NAAQS) of 12 $\mu g/m^3$ (at or above 12 $\mu g/m^3$ vs. below). Log-binomial models were then run with an interaction term between carrier status and dichotomized air pollution, and adjusting for sex, race/ethnicity, and neighborhood poverty level from CES 3.0 measured as the percent of the census tract below two times the federal poverty threshold. Interaction term *p*-values below 0.2 were considered statistically significant. Additionally, risk ratios and 95% CIs were computed within air pollution strata.

Asthma control analyses were conducted among asthmatics using negative binomial regression models with years of follow-up as an offset term to estimate incidence rate ratios (IRRs) and 95% CIs for associations between carrier status and asthma exacerbation rate or SABA prescription rate.

2.6 | Sensitivity analyses

There is no consensus on a medical claims-based definition of persistent asthma.[23] Therefore, we assessed the robustness of our results using two alternative definitions of asthma: (1) at least one EOC with an asthma primary diagnosis or (2) at least one EOC with an asthma primary diagnosis and at least one inhaled corticosteroid (ICS) prescription. The former was selected to minimize missed cases and the latter to define persistent pediatric asthma based on treatment guidelines.[17] ICS prescriptions were identified in the same way as SABAs.

All statistical analyses were conducted in SAS software version 9.4. Geocoding of maternal addresses was performed in ArcGIS version 10.7. The study was approved by the California Health and Human Services Agency Committee for Protection of Human Subjects (Project #2018-182), CDPH's Vital Statistics Advisory Committee (Order #18-07-0151), and California Biobank Program (Request ID: 1276).

3 | RESULTS

3.1 | Study population

The study group distribution remained consistent throughout the exclusion steps, resulting in 5746 subjects for analysis, including 941 carriers (Figure 1). Study population characteristics are presented in Table 1. Subjects were followed for an average of 43 person-months. The percentage of females was greatest among carriers, followed by High IRT controls and Low IRT controls. Carriers included a greater proportion of non-Hispanic White subjects compared to High IRT controls and Low IRT controls, whereas High IRT controls included a greater proportion of non-Hispanic Black subjects than Low IRT controls and carriers. F508del was the most prevalent panel variant in carriers.

3.2 | Risk of persistent asthma

Asthma prevalence ranged from 6.1% to 8.4% in the three study groups (Table 2). Adjustment for race/ethnicity and sex generally attenuated results when low IRT controls were in the comparison group and had the inverse effect when high IRT controls were in the comparison group. Carriers appeared to be at greater risk of asthma compared to Low IRT controls, with a nearly significant adjusted risk ratio (aRR) of 1.29 (95% CI: 0.98, 1.69; $p < 0.1$). When dividing

TABLE 1 Overall and study group-specific characteristics of a cohort of children enrolled in California Medicaid after the fifth birthday

	Low IRT	High IRT	*CFTR* carriers	Total
N	2303	2502	941	5746
Sex (%)				
Male	50.4	48	44.1	48.3
Female	49.6	52	55.9	51.7
Race (%)				
NH White	16.9	14.9	31.8	18.5
NH Black	10.7	29.3	16.3	19.7
Hispanic	62.6	49	48.1	54.3
NH Asian/Other	9.8	6.7	3.8	7.5
Birth year, median (IQR)	2010 (2009–2012)	2010 (2009–2012)	2010 (2009–2012)	2010 (2009–2012)
Age at last month of follow-up, years, mean (range)	8.7 (5–12)	8.7 (5–12)	8.8 (5–12)	8.7 (5–12)
Follow-up after fifth birthday, person-months, median (IQR)	45 (27–65)	41 (26–61)	42 (27–61)	43 (27–62)
Immunoreactive trypsinogen, μg/L, mean (SD)	22.2 (10.9)	81.3 (40.2)	82.6 (34.7)	57.8 (42.4)
$PM_{2.5}$, $\mu g/m^3$, mean (SD)[a,b]	11.2 (2.8)	11.0 (2.9)	10.7 (3.2)	11.0 (2.9)
Poverty, %, mean (SD)[a,c]	48.5 (19.0)	48.7 (19.1)	46.1 (18.7)	48.2 (19.0)
Prevalent *CFTR* panel variants, %				
F508del (c.1521_1523delCTT)	-	-	57.8	-
G542X (c.1642G>T)	-	-	6.2	-
3120+1G>A (c.2988+1G>A)	-	-	3.5	-
F311del (c.933_935delCTT)	-	-	3.5	-
G551D (c.1652G>A)	-	-	2.4	-
S549N (c.1646G>A)	-	-	2.3	-
3849+10kbC>T (c.3717+12191C>T)	-	-	2.2	-
3876delA (c.3744delA)	-	-	2.0	-
Others (28 different variants)	-	-	20.1	-

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator gene; IQR, interquartile range; IRT, immunoreactive trypsinogen; NH, non-Hispanic; PM2.5, particulate matter with aerodynamic diameter less than 2.5 microns; SD, standard deviation.

[a]Sample size reduced due to missing values. *N* in low IRT, high IRT, carriers, and total, respectively: 2295, 2498, 921, and 5714.

[b]CalEnviroScreen version 3.0 $PM_{2.5}$ indicator, a 3-year average (2011–2013) of census tract-level annual mean $PM_{2.5}$ estimates from a spatial interpolation model.

[c]CalEnviroScreen version 3.0 poverty indicator, percent of census tract population living below two times the federal poverty threshold (5-year American Community Survey estimate, 2011–2015).

carriers into those with single versus multiple variants, a greater risk ratio was observed for the single variant subgroup (aRR = 1.37, 95% CI: 1.03, 1.82) than the multiple variant subgroup (aRR = 0.95, 95% CI: 0.52, 1.71). When using CFTR2 categories, carriers with no additional variants in *trans* had a greater asthma risk (aRR=1.43, 95% CI: 1.07, 1.91) compared to carriers with at least one additional variant in *trans* (aRR=0.91, 95% CI: 0.54, 1.53). When analyzed individually, CFTR2 categories for additional variants (varying clinical consequence, unknown significance, non-CF-causing) in *trans* had similarly diminished risks (data not shown). The carrier subgroups at

TABLE 2 Unadjusted and adjusted risk ratios comparing asthma prevalence between *CFTR* carrier groups and two referent groups, Low IRT and High IRT, in a cohort of children enrolled in California Medicaid after the fifth birthday

	Asthma prevalence (%)	RR (95% CI)	aRR[a] (95% CI)
Low IRT	6.1	ref.	ref.
Carrier	8.2	1.34 (1.02, 1.75)**	1.29 (0.98, 1.69)*
High IRT	8.4	ref.	ref.
Carrier	8.2	0.97 (0.76, 1.25)	1.08 (0.83, 1.39)
Low IRT	6.1	ref.	ref.
One CF-causing variant	8.8	1.43 (1.08, 1.89)**	1.37 (1.03, 1.82)**
One CF-causing and other variants	5.9	0.96 (0.53, 1.74)	0.95 (0.52, 1.71)
High IRT	8.4	ref.	ref.
One CF-causing variant	8.8	1.04 (0.80, 1.36)	1.15 (0.88, 1.50)
One CF-causing and other variants	5.9	0.70 (0.39, 1.26)	0.79 (0.44, 1.42)
Low IRT	6.1	ref.	ref.
≥1 variant on a single allele	9.2	1.50 (1.12, 1.99)**	1.43 (1.07, 1.91)**
≥1 variant on both alleles	5.7	0.93 (0.56, 1.56)	0.91 (0.54, 1.53)
High IRT	8.4	ref.	ref.
≥1 variant on a single allele	9.2	1.09 (0.83, 1.43)	1.20 (0.91, 1.58)
≥1 variant on both alleles	5.7	0.68 (0.41, 1.13)	0.76 (0.45, 1.26)
Low IRT	6.1	ref.	ref.
F508del/T7 or (TG)11T5[b]	9.5	1.56 (1.13, 2.15)**	1.52 (1.10, 2.12)**
Other carriers	6.8	1.11 (0.77, 1.61)	1.07 (0.74, 1.54)
High IRT	8.4	ref.	ref.
F508del/T7 or (TG)11T5[b]	9.5	1.14 (0.84, 1.54)	1.26 (0.92, 1.72)
Other carriers	6.8	0.81 (0.57, 1.16)	0.90 (0.63, 1.30)

Note: *$p < 0.1$; **$p < 0.05$.

Abbreviations: aRR, adjusted risk ratio; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator gene; CI, confidence interval; IRT, immunoreactive trypsinogen; RR, risk ratio

[a]Derived from log-binomial models adjusted for race/ethnicity and sex.

[b]In some cases, subjects may have other variants in *cis* or *trans*.

greatest risk of asthma were (TG)11T5 or T7 in *trans* with an F508del variant (aRR=1.52, 95% CI: 1.10, 2.12). In a separate analysis of children with an F508del panel mutation, other (TG)mTn genotypes, 9 T, (TG)12T5, and (TG)13T5, in *trans* had the lowest risks of asthma (data not shown). Associations were similar but attenuated when High IRT controls were used as the referent group.

3.3 | Air pollution effect modification

Air pollution effect modification analyses were conducted on the subgroup with the greatest identified risk, F508del/T7 or (TG)11T5 (Figure 2). When Low IRT controls were the referent group, the association of F508del/T7 or (TG)11T5 with asthma prevalence was higher in areas with $PM_{2.5}$ levels at or above the NAAQS (aRR = 1.80, 95% CI: 1.09, 2.96) compared to areas below the NAAQS (aRR=1.37, 95% CI: 0.89, 2.10), though this difference was not statistically significant ($p_{interaction} > 0.2$) (data not shown). When High IRT controls were the referent group, associations tended to be lower in areas with increased air pollution ($p_{interaction} > 0.2$).

3.4 | Asthma control

We also conducted asthma control analyses in the F508del/T7 or (TG)11T5 group. Adjusted exacerbation rates among F508del/T7 or (TG)11T5 were similar to Low IRT controls (aIRR = 1.07, 95% CI: 0.66, 1.74) (Table 3). A weak association was observed when High IRT controls were used as the referent group (aIRR = 1.30, 95% CI: 0.82, 2.05). The SABA prescription rate was non-significantly elevated in

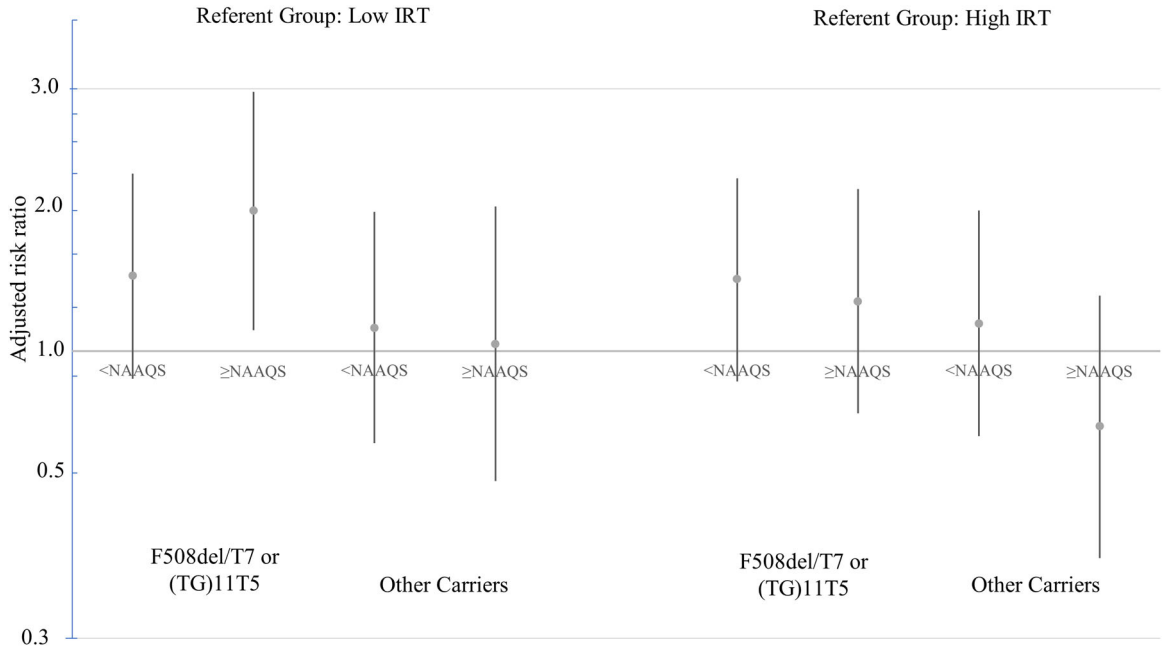

FIGURE 2 Adjusted risk ratios and 95% confidence intervals for associations between *CFTR* carrier group and asthma prevalence among a cohort of children enrolled in California Medicaid after the fifth birthday, with birth address in an area at or above versus below the primary $PM_{2.5}$ National Ambient Air Quality Standard (12 μg/m^3), adjusted for race/ethnicity, sex, and neighborhood poverty level. CFTR, cystic fibrosis transmembrane conductance regulator gene; NAAQS, National Ambient Air Quality Standard; $PM_{2.5}$, particulate matter with aerodynamic diameter less than 2.5 microns

the F508del/T7 or (TG)11T5 group compared to Low IRT controls (aIRR=1.14, 95% CI: 0.85, 1.53) (Table 4).

3.5 | Sensitivity analyses

The increased asthma risk in the F508del/T7 or (TG)11T5 group compared to the Low IRT referent group remained when asthma was defined as one or more primary diagnoses (aRR=1.30, 95% CI: 1.02, 1.66) (Tables S1). The association was similar and nearly statistically significant when asthma was defined as at least one primary diagnosis of asthma and at least one ICS prescription (aRR=1.38, 95% CI: 0.95, 2.02; $p < 0.1$) (Table S2). Exacerbation estimates were null when at least one diagnosis was used to define asthma (Table S3). The exacerbation rate was higher in the F508del/T7 or (TG)11T5 group compared to Low IRT controls when one diagnosis and one ICS prescription were used as the definition, with the estimate approaching statistical significance (aIRR=1.59, 95% CI: 0.98, 2.58; $p < 0.1$) (Table S4). SABA prescription rates were elevated in the F508del/T7 or (TG)11T5 group compared to either referent group, under either asthma definition (Tables S5 S6). When at least one asthma diagnosis was used to define asthma, the effect of being in the other carriers group was significantly reduced in areas with higher air pollution compared to areas with lower air pollution, whether Low IRT ($p_{interaction} = 0.15$) or High IRT ($p_{interaction} = 0.02$) was used as the referent group (Figure S1); this was not observed under the asthma and ICS definition (Figure S2).

4 | DISCUSSION

In this California Medicaid population, we found that carriers of a single *CFTR* CF-causing variant were at higher risk of persistent childhood asthma, consistent with most previous studies.[7] Contrary to our hypothesis, we did not find evidence that a second *CFTR* variant in *trans* phase increased this risk; rather, these subjects had a comparable or lower risk of asthma compared to the referent groups. Using CFTR2 to categorize these variants into varying clinical consequence, non-CF-causing, or as yet unknown significance, did not yield additional specificity to this finding, and suggests that the relationship between CFTR function and asthma risk is not monotonic.

When specific genotypes were explored, including the Intron 9 (TG)mTn Poly-Variant Tract, the highest risk was found in subjects who had an F508del variant on one allele and a (TG)11T5 or T7 on the second allele. Interestingly, this subgroup comprises over half of the CF carriers in our sample, thus strongly influencing the overall carrier effect. The literature suggests that having an F508del variant on one allele confers 1% CFTR wildtype function[19] and having a (TG)11T5 or T7 on the second allele confers 24%–44% function,[18] for a total expected function of 25%-45%. Other carrier subjects with a lower risk of asthma are a mixture of genotypes with both higher (e.g., F508del with 9T in *trans*, 48%) and lower (e.g., F508del with (TG)12T5 in *trans*, 15%) CFTR functioning, and likely other genotypes that fall outside of the 25%–45% functional range. Given the current minimal state of knowledge on *CFTR* genotype function in carriers

TABLE 3 Unadjusted and adjusted incidence rate ratios comparing asthma exacerbation rates among *CFTR* carriers with an F508del variant and a 7T or 5T-11(TG) TG-Poly T tract in *trans* and other carriers, versus Low IRT and High IRT referent groups, in a cohort of children enrolled in California Medicaid after the fifth birthday

	N	Follow-up (person-years)	Exacerbations (N)	Average rate (exacerbations per 10 person-years)	IRR (95% CI)	aIRR[a] (95% CI)
Low IRT	140	665	167	2.51	ref.	ref.
F508del/T7 or (TG)11T5[b]	45	211	56	2.65	1.14 (0.70, 1.84)	1.07 (0.66, 1.74)
Other carrier	32	157	42	2.68	1.00 (0.58, 1.71)	0.92 (0.53, 1.58)
High IRT	210	984	232	2.36	ref.	ref.
F508del/T7 or (TG)11T5[b]	45	211	56	2.65	1.20 (0.76, 1.89)	1.30 (0.82, 2.05)
Other carrier	32	157	42	2.68	1.06 (0.63, 1.77)	1.15 (0.69, 1.93)

Abbreviations: aIRR, adjusted incidence rate ratio; CI, confidence interval; CFTR, cystic fibrosis transmembrane conductance regulator gene; IRR, incidence rate ratio; IRT, immunoreactive trypsinogen.

[a]Derived from negative binomial models adjusted for race/ethnicity and sex.

[b]In some cases, subjects may have other variants in *cis* or *trans*.

TABLE 4 Unadjusted and adjusted incidence rate ratios comparing SABA prescription rates among *CFTR* carriers with an F508del variant and a T7 or (TG)115T TG-Poly T tract in *trans* and other carriers, versus Low IRT and High IRT referent groups, in a cohort of children enrolled in California Medicaid after the fifth birthday

	N	Follow-up (person-years)	SABA prescriptions (N)	Average rate (prescriptions per 10 person-years)	IRR (95% CI)	aIRR[a] (95% CI)
Low IRT	140	665	1225	18.42	ref.	ref.
F508del/T7 or (TG)11T5[b]	45	211	425	20.14	1.17 (0.87, 1.56)	1.14 (0.85, 1.53)
Other carrier	32	157	414	26.37	1.05 (0.76, 1.47)	1.06 (0.76, 1.48)
High IRT	210	984	1936	19.67	ref.	ref.
F508del/T7 or (TG)11T5[b]	45	211	425	20.14	1.08 (0.84, 1.38)	1.12 (0.88, 1.44)
Other carrier	32	157	414	26.37	0.98 (0.74, 1.30)	1.03 (0.77, 1.38)

Abbreviations: aIRR, adjusted incidence rate ratio; CFTR, cystic fibrosis transmembrane conductance regulator gene; CI, confidence interval; IRR, incidence rate ratio; IRT, immunoreactive trypsinogen; SABA, short-acting beta agonist.

[a]Derived from negative binomial models adjusted for race/ethnicity and sex.

[b]In some cases, subjects may have other variants in *cis* or *trans*.

and the low prevalence of other non-F508del variants, we refrained from grouping these other variants according to percent wildtype function in this analysis. We also used a simplified approach to summing allelic percent function to generate the total expected genotype percent function which warrants confirmation by others. It is possible that the non-monotonic risk observed in this study may be the result of selection bias in the group of children with more than one *CFTR* variant because some of them were diagnosed with CF (known to have asthma-like symptoms[24]) and thus were removed from the analysis.

Although the sample size was small for investigating interactions, there was suggestive evidence of effect modification by a crude measure of air pollution in this low-income population. This finding is consistent with the importance of environmental factors in the pathogenesis of asthma, especially among those with high-risk *CFTR* genotypes.

This study faced several limitations. We did not conduct genotyping in the Low IRT control group and only conducted 40-panel variant testing in the High IRT control group, potentially resulting in the inclusion of *CFTR* carriers in those groups. The prevalence of CF cases and carriers increases as newborn IRT levels increase,[13] so the Low IRT control group is less likely to include carriers than the High IRT control group. Although the High IRT control group was screened for 40 common CF-causing variants, this does not rule out the presence of other prevalent *CFTR* variants, such as R117H [c.350G>A], which were not on the panel. This lack of a clean referent group likely diminished the strength of any associations found, especially when High IRT controls were the referent group. This study had limited power to detect interactions between genotype and air pollution due to low variability in air pollution exposure and misclassification of air pollution exposure due to possible relocation out of the birth address census tract during pregnancy or early childhood. The generalizability of our findings may be limited as our study population consisted primarily of low-income, non-Hispanic Black, and Hispanic Medicaid enrollees.

While our epidemiologic study findings are consistent with a meta-analysis showing that *CFTR* variant carriers are at increased risk of asthma,[7] they suggest that this risk is not monotonic, but rather accentuated in a subgroup with 25%–45% CFTR wildtype function (e.g., F508del in *trans* with (TG)11T5 or T7). The recent observation that carriers are at higher risk of over 50 other conditions[8] suggests that CFTR dysfunction may have multiple mechanisms of action. We encourage others to attempt replication of our findings and to explore the possible mechanisms of action. Overall, these results support further research regarding the clinical benefit of *CFTR* testing in asthmatics unresponsive to standard asthma treatments, and have implications for the design of CF screening programs, referrals, educational materials, and the study of alternative treatment options such as CFTR modulators and correctors for CFTR-related asthma.

AUTHOR CONTRIBUTIONS

Ruwan Thilakaratne: Formal analysis (lead), Methodology (lead), Writing – original draft (equal), Writing – review & editing (lead). **Steve Graham**: Data curation (lead), Formal analysis (supporting), Methodology (supporting), Validation (lead), Writing – review & editing (supporting). **John Moua**: Formal analysis (supporting), Methodology (supporting), Writing – review & editing (supporting). **Caitlin Jones**: Data curation (supporting), Formal analysis (supporting), Writing – review & editing (supporting). **Caroline Collins**: Data curation (supporting), Writing – review & editing (supporting). **Jennifer Mann**: Formal analysis (supporting), Methodology (supporting), Validation (supporting), Writing – review & editing (supporting). **Stanley Sciortino**: Data curation (supporting), Formal analysis (supporting), Writing – review & editing (supporting). **Jacklyn Wong**: Conceptualization (supporting), Data curation (supporting), Methodology (supporting), Project administration (supporting), Writing – original draft (equal), Writing – review & editing (supporting). **Martin Kharrazi**: Conceptualization (lead), Data curation (supporting), Formal analysis (lead), Methodology (lead), Project administration (lead), Supervision (lead), Writing – original draft (equal), Writing – review & editing (lead).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Participant data cannot be made available due to legal and ethical requirements restricting access to individual-level data from the California newborn screening program.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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