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Authors

Izquierdo, Manuel E
Marion, Chad R
Moore, Wendy C
et al.

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DNA Sequencing Analysis of Cystic Fibrosis Transmembrane Regulator Gene Identifies Cystic Fibrosis-Associated Variants in the Severe Asthma Research Program.

Manuel E. Izquierdo[1], **Chad R. Marion**[2], **Wendy C. Moore**[3], **K. Raraigh**[4], **Jennifer L Taylor-Cousar**[5], **Gary R. Cutting**[6], **E. Ampleford**[7], **Gregory A. Hawkins**[8], **Joe Zein**[9], **M. Castro**[10], **Loren C. Denlinger**[11], **Serpil C. Erzurum**[12], **John V. Fahy**[13], **Elliot Israel**[14], **Nizar N. Jarjour**[15], **David Mauger**[16], **Bruce D. Levy**[14], **Sally E. Wenzel**[17], **Prescott Woodruff**[18], **Eugene R. Bleecker**[19], **Deborah A. Meyers**[20], **Victor E. Ortega**[21]

[1]Wake Forest Baptist Medical Center, Winston Salem, NC, United States

[2]Internal Medicine, Wake Forest University, Winston Salem, NC, United States

[3]Wake Forest Sch of Med, Winston-Salem, NC, United States

[4]Johns Hopkins, Baltimore, MD, United States

[5]Department of Medicine and Pediatrics, National Jewish Health, Denver CO

[6]Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD, United States,

[7]Internal Medicine, Wake Forest School of Medicine, Winston-Salem, NC, United States

[8]Center for Precision Medicine, Wake Forest Baptist Health, Winston Salem, NC, United States

[9]Departments of Inflammation and Immunity, Cleveland Clinic, Cleveland, OH, United States

[10]Pulmonary Critical Care & Sleep Med, Univ of Kansas, Kansas City, KS, United States

[11]Department of Medicine, University of Wisconsin-Madison, Madison, WI, United States

[12]Cleveland Clinic Foundation, Cleveland, OH, United States

[13]Pulmonary and Critical Care Medicine, Univ of California At San Francisco, San Francisco, CA, United States

[14]Brigham and Womens Hosp, Boston, MA, United States

[15]University of Wisconsin Hospitals & Clinics, Madison, WI, United States

[16]Pennsylvania State University, University Park, PA, United States

[17]Professor of Med PACCM, University of Pittsburgh, Pittsburgh, PA, United States

[18]Medicine, University of California, San Francisco, San Francisco, CA, United States

[19]Medicine, University of Arizona, Tucson, AZ, United States

Corresponding Author: Victor E. Ortega, MD, PhD, ATSF, Senior Associate Consultant, Department of Internal Medicine, Division of Respiratory Diseases, Center for Individualized Medicine, Mayo Clinic, 13400 E. Shea Boulevard, Scottsdale, Arizona, Ortega.Victor@mayo.edu, Phone: (480) 301 9432.

[20]Genetics, Genomics, and Precision Medicine, University of Arizona, Tucson, AZ, United States

[21]Mayo Clinic, Scottsdale, AZ, United States.

Abstract

Background: Heterozygote carriers of potentially pathogenic variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene have increased asthma risk. However, the frequency and impact of *CFTR* variation among individuals with asthma is unknown.

Objective: To determine whether potentially pathogenic *CFTR* variants associate with disease severity and whether individuals with two potentially pathogenic variants exist in a severe asthma-enriched cohort .

Methods: We analyzed sequencing data spanning a 190.5Kb region of *CFTR* in participants from the Severe Asthma Research Program (SARP1–3). Potentially pathogenic, rare *CFTR* variants (frequency<0.05) were classified as CF-causing or of varying clinical consequences (VVCC) (CFTR2.org). Regression-based models tested for association between *CFTR* genotypes (0–2 potentially pathogenic variants) and severity outcomes.

Results: Of 1401 participants, 9.5% (134) had one potentially pathogenic variant, occurring more frequently in non-Hispanic white (NHW, 10.1% [84 of 831]) compared to African American individuals (AA, 5.2% [22 of 426]). We found ≥2 potentially pathogenic *CFTR* variants in 1.4% (19); 0.5% (4) of NHW and 2.8% (12) of AA. Potentially pathogenic *CFTR* variant genotypes (≥1 or ≥2 variants) were not cumulatively associated with lung function or exacerbations. In NHW, we found three F508del compound heterozygotes with F508del and a VVCC (two 5T;TG12[c.1210–11T>G] and one Arg1070Trp) and a homozygote for the VVCC, 5T;TG12.

Conclusions: We found potentially pathogenic *CFTR* variants within a severe asthma-enriched cohort, including three compound heterozygote genotypes variably associated with CF in NHW individuals. These findings provide the rationale for *CFTR* sequencing and phenotyping of CF-related traits in individuals with severe asthma.

Introduction

Cystic fibrosis (CF) is a disease of the airways that occurs most commonly in individuals of European descent, but can occur in individuals of any race or ethnicity. CF is caused by variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene and characterized by bronchiectasis with a subgroup of individuals experiencing overlapping features with a much more common airways disease, asthma. A historical study showed that up to 50% of individuals with CF had airway reactivity thought to be related to the severity of lung disease. More recently, the 2020 US CF Foundation Patient Registry has reported the prevalence for CF-asthma overlap to be 31.1% which contrasts to general population estimates of 7.8%[(1)]. Epidemiologic data published in general populations and resulting meta-analyses have also demonstrated an increased risk of asthma in heterozygous carriers of pathogenic *CFTR* variants.[(2)] Most of this risk has been attributed to heterozygote carriers of the common pathogenic CFTR variant, c.1521_1523delCTT (p.Phe508del; legacy: F508del), in whom lung function impairment has also been reported among those with asthma from a general population.[(3)] The biologic plausibility of *CFTR* as an asthma

risk modifier locus is supported by animal models reporting increased airway responsiveness independent of mucous plugging and inflammation. The limitations of these prior studies are that they (1) primarily used targeted genotyping rather than sequencing which can miss numerous potentially pathogenic variants, (2) the diagnosis of asthma was based on a physician's diagnosis which can be prone to misclassification, and (3) that these studies focused on asthma risk and not severity. We report a sequencing-based study of the CFTR locus to identify and evaluate the clinical impact of potentially pathogenic CFTR variation in a multi-racial/ethnic asthma cohort enriched for severe disease, the NHLBI-sponsored Severe Asthma Research Program (SARP1–3). Furthermore, because highly effective therapies targeting the CFTR protein (CFTR modulators) are approved and available, identification of pathogenic CFTR variants in this population may have important therapeutic implications.[4]

Results

SARP participants with asthma (n= 1401, SARP1–3) had whole genome sequencing(after consent was attained) through the NHLBI-sponsored TOPMed program. We extracted sequencing data on functional variation spanning a 190.5Kb region of *CFTR* (hg19 position chromosome 7:117119040–117309560). Potentially pathogenic, low frequency-to-rare *CFTR* variants (allele frequency<0.05) were classified as (1) CF-causing based on the CFTR2 database (cftr2.org), (2) having varying clinical consequences on CF risk (VVCC) based on CFTR2, and (3) likely pathogenic based on the American College of Medical Genetics (ACMG)(acmg.net) assuming a recessive model (Figure 1). The minor allele frequency of these potentially pathogenic CFTR variants and regression-based models were stratified by self-reported racial/ethnic group (African American and non-Hispanic whites). Regression-based models tested for associations of potentially pathogenic genotypes with clinical outcomes of interest including the comparison between (1) individuals with no identifiable potentially pathogenic variation versus 1 potentially pathogenic variant, (2) one or more variants, or (3) 2 potentially pathogenic variants using collapsing-based burden tests. Variant-specific models also compared individuals with and without F508del and no other potentially pathogenic variants (i.e. F508del heterozygotes).

Of 1401 total participants, 9.5% (134) were carriers of one potentially pathogenic *CFTR* variant and these carriers were more likely to be non-Hispanic white (NHW, 10.1% [84 of 831]) when compared to African American individuals (AA, 5.2% [22 of 426]). The most frequent potentially pathogenic *CFTR* variant identified was F508del, found in 30 individuals, the majority of whom were NHW (3.5% [29 of 831]). We found ≥2 potentially pathogenic *CFTR* variants in 1.4% (19) of total participants which occurred more frequently in African American (2.8%, n=12) compared to NHW individuals (0.5%, n= 4). Potentially pathogenic *CFTR* variant genotypes (none versus ≥1 or ≥2 potentially pathogenic variants), including F508del, were not cumulatively associated with lung function measures or exacerbations requiring corticosteroid bursts, ED visits, or hospitalizations. In four NHW individuals, we found three with F508del compound heterozygosity with a VVCC: two c.1210–11T>G(legacy: 5T;TG12), and one with c.3208C>T(p.Arg1070Trp; legacy: R1070W; a CFTR modulator therapy-eligible variant) and an individual homozygous for the VVCC 5T;TG12. All four individuals were females with a reduced pre-bronchodilator

forced expiratory volume in 1 second (FEV1) percentage predicted (Table 1). All 12 AA with two potentially pathogenic variants were carriers of the cis-variants c.220C>T (p.Arg74Trp; legacy: R74W) and c.3808G>A (p.Asp1270Asn; legacy: D1270N), both FDA-approved targets for *CFTR* modulator therapy and highly likely to be occurring in *cis* and in full linkage disequilibrium. The CF-causing variants we identified in NHW and AA, especially F508del, had a markedly higher minor allele frequencies (MAF) in SARP participants compared to reference general populations (Table 2).

Discussion

The increased allele frequency of potentially pathogenic CFTR variation we report may be partially attributed to the smaller sample size of the deeply characterized SARP cohort, but is consistent with prior reports of a higher frequency of potentially pathogenic *CFTR* variants, especially F508del, in individuals with asthma from the general population.[2, 3] In NHW individuals from SARP, the allele frequency for F508del was >5-fold higher than that reported in European general populations (Table 2).[5] Although we hypothesized biologic plausibility, we found no evidence that *CFTR* variation influences asthma severity. The lack of an association could result from a lack of pathogenicity data. For example, we identified 91 coding variants in the exons of *CFTR* of which we were only able to find 62 catalogued in CFTR2 or by ACMG. Another potential reason for a lack of a severity association is that determinants of asthma are heterogeneous and complex stemming from numerous genes potentially masking any *CFTR*-specific effects in SARP.

Twelve African American individuals carried two African descent *cis* potentially pathogenic variants in perfect linkage disequilibrium that were both *CFTR* variant targets for CFTR modulators. This phenomenon of potentially pathogenic cis-occurring variants has been described previously in the literature and results in a complex allele with additive effects for these and other European descent variants associated with CF or congenital bilateral absence of the vas deferens(CBAVD).[6] Interestingly, when associated with CBAVD or mild CF there is usually an additional in cis variant (c.601G>A; p.Val201Met; legacy: V201M) that we did not find in these individuals.[6] Even though we found a smaller number of potentially pathogenic *CFTR* variants in AA individuals, we found a substantial number of African ancestry-specific variants in AA that we were unable to categorize because current databases primarily consist of data from European descent white individuals. Of the variants we identified with confirmed or potential pathogenicity, eight were found in AA while 21 were found in NHW individuals. A similar lack of identifiable pathogenic variation was described in both individuals with clinical CF from Puerto Rico and the Dominican Republic.

Four non-Hispanic white individuals with asthma of varying severity were either homozygous or compound heterozygous for potentially pathogenic CFTR variation of which three would have qualified for CFTR modulator therapy in the appropriate clinical setting. None of the potentially pathogenic genotypes we identified consisted of two CF-causing variants, but always had at least one VVCC. In the absence of concomitant demonstration of measured CFTR dysfunction, these individuals would not meet criteria for a confirmed diagnosis of CF. However, the clinical significance of these *CFTR* genotypes might relate

to CF masquerading as asthma in individuals with CF-asthma overlap or as an asthma disease-modifying genotype in the absence of CF, and further evaluation for CF would be warranted in such individuals. The analysis of next-generation *CFTR* sequencing data in larger asthma and general populations will be required and are currently underway (https://topmed.nhlbi.nih.gov/) to further characterize and confirm our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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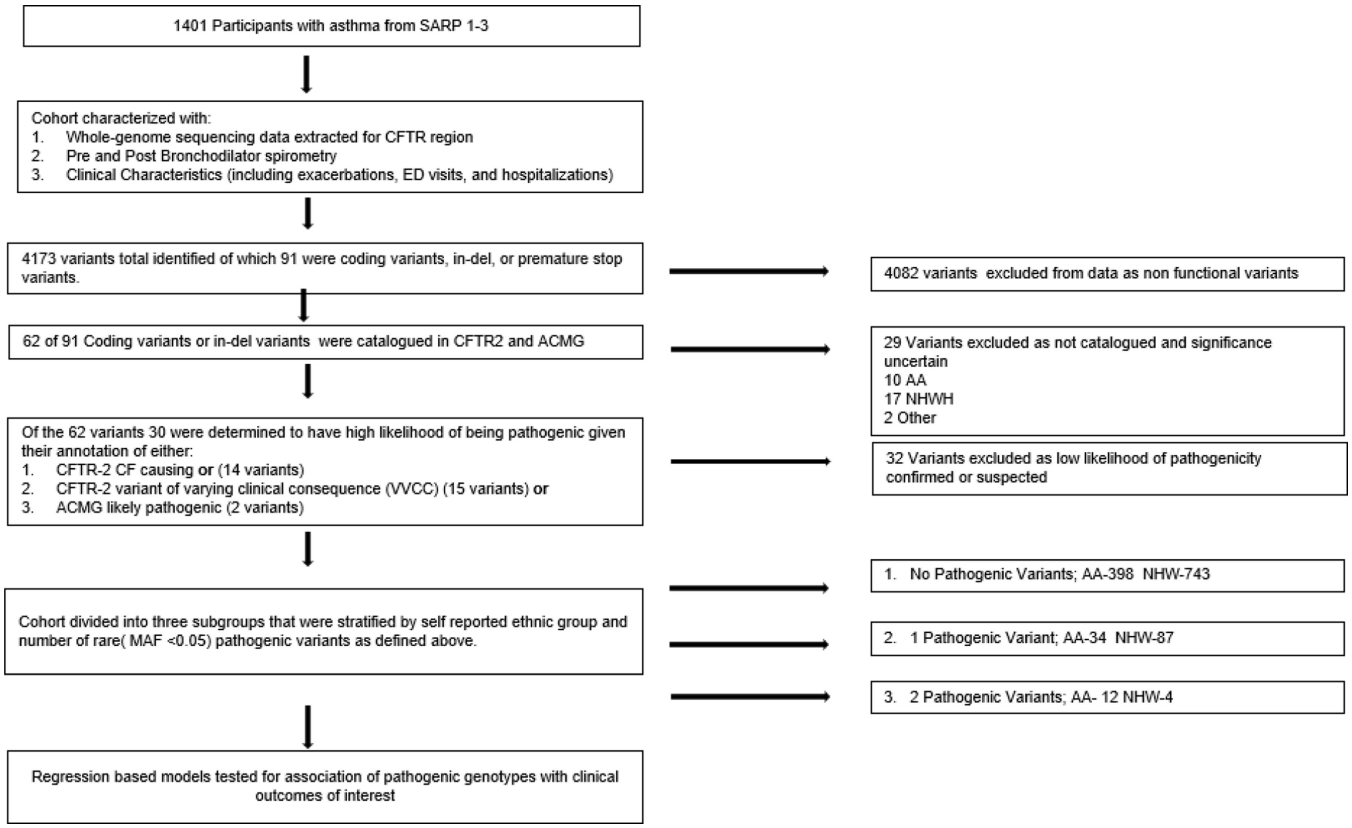

Figure 1. Flow diagram for methods

Flow diagram for characterization, selection, and analyses of individuals. Definition of abbreviations are as follows: CFTR=cystic fibrosis transmembrane regulator gene, CFTR2=clinical and functional translation of CFTR project, SARP=severe asthma research program, ED=emergency department, in-del-insertion deletion, CF-C=cystic fibrosis causing, VVCC=variants of varying clinical consequences, ACMG=American College of Medical Genetics, MAF=Minor allele frequency, AA=African American, NHW=non-Hispanic white.

Table 1.

Characteristics of Individuals with Two Potentially Pathogenic CFTR Variants.

CFTR Genotype	F508del/5T;TG12	5T;TG12/5T;TG12	F508del/5T;TG12	F508del/Arg1070Trp
Age (years)	30	24	69	59
Age of asthma onset (years)	22	6	6	5
Sex	Female	Female	Female	Female
Pre-bronchodilator FEV1% predicted	65%	77%	51%	77%
Post-bronchodilator FEV1% predicted	78%	113%	63%	NA
Pre-bronchodilator FEV1/FVC	0.72	0.58	0.67	0.88
Treated with LABA+ICS	Yes	No	Yes	Yes
Hospitalization last 12 months	No	No	No	No
Lifetime Hospitalization	No	NA	Yes	No
ER visit last 12 months	No	Yes	No	No
Unscheduled outpatient visit last 12 months	Yes	Yes	Yes	Yes
Corticosteroid burst last 12 months	No	No	No	No
Diabetes	No	No	No	Yes

Definitions of abbreviations are as follows: CFTR=cystic fibrosis transmembrane regulator gene, LABA=long acting beta agonist, ICS=inhaled corticosteroid, ER=emergency room, NA=No information available, FEV1=forced expiratory volume in 1 second, FVC=forced vital capacity.

Table 2.

CFTR Functional Variants by Ethnic Group in SARP and a General Population.

Legacy Name (cDNA)	Database annotation and pathogenicity	SARP AA	SARP NHW	AA	Europeans
394delTT (c.262_263del)	CFTR-2 CF-C	0	0.00059	0	0.00014
621+1G->T(c.489+1G>T)	CFTR2 CF-C	0	0.00059	0.0002	0.00016
F191V (c.571T>G)	CFTR2 CF-C	0.0012	0	0.0004	0
R347P(c.1040G>C)	CFTR2 CF-C	0	0.00059	0	0.00005
A455E (c.1364C>A)	CFTR2 CF-C	0	0.00059	0	0.0001
F508del (c.1521_1523del)	CFTR2-CF-C	0	0.018	0.0015	0.0031
1717–1G->A(c.15851G>A)	CFTR2 CF-C	0.0012	0	0.0002	0.00021
G551D (c.1652G>A)	CFTR2 CF-C	0	0.0012	0.0004	0.00047
R851X (c.2551C>T)	CFTR2-CF-C	0	0.00059	0	0.00001
2789+5G->A(c.2657+5G>A)	CFTR-2 CF-C	0	0.00059	0	0.0001
*__L1077P__ (c.3230T>C)	CFTR-2 CF-C	0	0	0	0.00002
*__W1098R__ (c.3292T>C)	CFTR-2 CF-C	0	0	0	0
W1282X (c.3846G>A)	CFTR2-CF-C	0	0.0012	0	0.00052
N1303K (c.3909C>G)	CFTR-2 CF-C	0	0.0012	0	0.00033
5T;TG12 (c.1210–11T>G)	CFTR-2 VVCC	0.015	0.018	0.0067	0.0154
R74W (c.220C>T)	CFTR-2 VVCC	0.019	0	0.0057	0.00014
R117H (c.350G>A)	CFTR-2 VVCC	0	0.003	0.0002	0.00253
621+3A->G (c.489+3A>G)	CFTR-2 VVCC	0	0.00059	0	0.00039
R334Q (c.1001G>A)	CFTR-2 VVCC	0	0.00059	0	0.00018
D443Y (c.1327G>T)	CFTR-2 VVCC	0	0.00059	0.0003	0.00033
G622D (c.1865G>A)	CFTR-2 VVCC	0.0023	0	0.0015	0
P750L (c.2249C>T)	CFTR-2 VVCC	0.0012	0.0012	0.0002	0.00077
L967S (c.2900T>C)	CFTR-2 VVCC	0	0.0024	0.0004	0.00127
Y1032C (c.3095A>G)	CFTR-2 VVCC	0	0.00059	0	0.00005
F1052V (c.3154T>G)	CFTR-2 VVCC	0	0.00059	0	0.00094
G1069R (c.3205G>A)	CFTR-2 VVCC	0	0.00059	0	0.00015
F1099L (c.3297C>A)	CFTR-2 VVCC	0.0012	0	0.0002	0.00002
D1270N (c.3808G>A)	CFTR-2 VVCC	0.014	0	0.0078	0.00005
1342–2A->C (c.1210–2A>C)	ACMG LP	0.	0.000595	0	0.0001
*__S589N__ (c.1766G>A)	ACMG LP	0	0	0	0

The minor allele Frequencies (MAF) are provided for confirmed and potentially pathogenic variants found with CFTR sequencing data in SARP. MAF for reference populations provided from the ALFA project in dbGap(https://www.ncbi.nlm.nih.gov/snp/). Definition of abbreviations are as follows: AA=African American, NHW=non-Hispanic white, CF-C=cystic fibrosis causing, VVCC=variants of varying clinical consequences. ACMG LP=American College of Medical Genetics-Likely Pathogenic, SARP-Severe Asthma Research Program.

*Variants with MAF of 0 in AA and NHW were found in “Other” ethnic group which is not listed.