CFTR mutations and phenotypic correlations in people with cystic fibrosis: a retrospective study from a single centre in south India

Sneha D. Varkki,[a] Rekha Aaron,[b] Aaron Chapla,[c] Sumita Danda,[b] Priyanka Medhi,[a] N. Jansi Rani,[d] and Grace R. Paul[e,*]

[a]Department of Paediatrics, Christian Medical College, Vellore, Tamil Nadu, India
[b]Department of Medical Genetics, Christian Medical College, Vellore, Tamil Nadu, India
[c]Department of Endocrinology, Christian Medical College, Vellore, Tamil Nadu, India
[d]Department of Biostatistics, Christian Medical College, Vellore, Tamil Nadu, India
[e]Division of Pulmonary and Sleep Medicine, Nationwide Children's Hospital, Columbus, OH, USA

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Summary

Background Emerging data reveal higher-than-expected prevalence of cystic fibrosis (CF) among non-European populations worldwide including in the Indian subcontinent. Systematic analyses of the *CFTR* mutation profile, and genotype-phenotype correlations among people with CF from south, east, or northeast India have not been reported before. We wanted to identify *CFTR* mutations in people with CF, and highlight novel variants, selective phenotypic correlations, and regional variances within India.

Methods A retrospective study was conducted at Christian Medical College, Vellore, India (single tertiary referral hospital) from September 2010 to August 2022, involving 120 people with CF from (i) four south Indian states (Tamil Nadu, Andhra Pradesh, Kerala, Karnataka), (ii) in and nearby regions of West Bengal, India and (iii) Bangladesh. Comprehensive *CFTR* mutation analyses were done by Next-Generation Sequencing, and variants were categorized per American College of Medical Genetics guidelines and compared with validated Locus-specific databases. Demographic characteristics, mutation profile, novel mutations, selective phenotype correlations, and regional variances were assessed.

Findings In 120 people with CF, 55 CFTR variants were identified, including six novel variants. F508del was the predominant mutation, yet with a lower allele frequency than reported among European populations (27% versus 70%). Phenotypic correlations suggested high mutational pathogenicity causing severe multi-organ morbidity, and death in 27%. Milder variants associated with pancreatic sufficiency were also evident in 23% of people with CF. Statistically significant regional variances were noted in genotype frequency, and clinical phenotype among people with CF from the two regions. Hotspot exons and introns that could potentially help create targeted mutation panels were identified.

Interpretation The identification of 55 different *CFTR* variants among 120 people with CF describes the diversity of mutations noted in India, while also revealing the challenges that providers may encounter in timely diagnosis and treatment of CF. However, these single-centre data have specific limitations and cannot be generalised to all people with CF from India or to those of non-European origin. Our data on regional *CFTR* mutations contribute to the emerging national registry on CF epidemiology in India, help formulate diagnostic and newborn screening algorithms, help optimise clinical care, and highlight urgency to improve access to life-changing modulator therapy.

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Keywords: CF; India; *CFTR* mutations; Genetics

*Corresponding author. Division of Pulmonary and Sleep Medicine, Nationwide Children's Hospital, 700 Children's Drive, Columbus, OH 43025, USA.
E-mail address: Grace.Paul@nationwidechildrens.org (G.R. Paul).

Research in context

Evidence before this study

Historically, CF has been described among European populations, however, emerging data reveal a high CF disease-burden in many low-income and middle-income countries, including India. As a systematic study to understand the CF genetic profile or the disease-burden has never been conducted in India, we report for the first time, the genetic analysis of 120 people with CF from areas of India and Bangladesh followed at a referral hospital in south India. We searched PubMed for all available data until July 2023, with the terms "cystic fibrosis (CF)", "*CFTR* mutation", "India", and "genetics". We restricted the search to articles published in English. The earliest report of CF from India was from 1967; however, overall, there is a paucity of data on this topic until recently published case reports and case series on CF epidemiology in India, especially over the past decade. The reported prevalence of CF in India based on immigrants in the USA and UK is 1/10,000 to 1/40,000, which we believe is a gross underestimation of the true epidemiology of CF in India. A recent report by Guo and colleagues indicated that India could be a 'hotspot' location with exceedingly high unrecognised CF burden. From a genetic perspective, even though F508del was the most noted mutation among persons with CF in South Asia, the allele frequency of F508del was only 27% compared to 85% among those of European ancestry. In two studies done in north India, the carrier frequency of F508del was estimated to be 1 in 238 in a cohort of 955 live births by cord blood sampling, and the carrier frequency of any pathogenic *CFTR* mutation was alarmingly high at 4.5% among 200 randomly selected healthy adults. Case reports and case series reporting *CFTR* mutation profiles from specific medical centres or regions in India were reviewed to understand regional variances in *CFTR* profile.

Added value of this study

This is the first study to describe the *CFTR* mutation profile of persons with CF, predominantly from south India, and from certain regions of east and north-east India. In this study, we identified 55 variants of the *CFTR* gene, including six novel variants. *CFTR* allele frequencies of known variants were compared with Locus-Specific DataBases from North America and France (CFTR2 and CFTR-France), and novel variants were annotated per the American College of Medical Genetics (ACMG) guidelines. Our data augment awareness of the existence of CF in India and help define CF genetic profiles of certain specific regions of the Indian subcontinent. The identification of 55 different *CFTR* variants among 120 people with CF describes the diversity of mutations noted in India, while also revealing the challenges that providers may encounter in timely diagnosis and treatment of CF. These data and interpretation reveal diagnostic and clinical implications in management of CF in India, especially as we highlight (i) the pathogenicity of the identified mutations, many with serious genotype-phenotype correlations, (ii) presence of modulator-eligible mutations, (iii) the diagnosis of CF by mutation analysis despite non-diagnostic sweat chloride levels, and (iv) potential role of targeted mutation panels for CF diagnosis. We also report for the first time, statistically significant regional variances in CF genotype and clinical phenotype among people with CF within India.

Implications of all the available evidence

There are several implications of our findings from epidemiologic, anthropologic, clinical, diagnostic, and ethical perspectives. Our data will (i) add to existing literature on CF in the Indian subcontinent by enabling a better understanding of the *CFTR* mutation profile in this region, (ii) help advocate for better access and affordability to *CFTR* genetic testing, (iii) help create targeted mutation panels for CF diagnosis that would be practical, cost-effective, and be of regional relevance, (iv) highlight modulator-eligible mutations and improve access and affordability to CF-specific therapies, (v) contribute to the global *CFTR* databases, and (vi) add to the emerging national CF registry. Our data will augment current advocacy efforts to improve the healthcare infrastructure for early CF diagnosis (by neonatal screening), and to provide basic CF care. Such improvement in healthcare infrastructure would reduce CF-related infant and early childhood morbidity and mortality, over time. We acknowledge that our data are from a single-centre and cannot be generalised to people with CF from India as a whole. Apart from direct clinical benefit, these data will also pique interest for further anthropologic studies and genetic profiling of CF as an autosomal recessive disease in a highly multi-ethnic and populous nation with high rates of consanguinity and emigration.

Introduction

Cystic Fibrosis (CF) is a life-limiting monogenic disease that occurs due to mutations in the Cystic Fibrosis Transmembrane regulator (*CFTR*) gene.[1] The *CFTR* gene spans 189 kilobases on chromosome 7,[2] encoding 1480 amino acids in the mature protein. In epithelial cells lining the airway and other organs, *CFTR* protein functions as a regulatory channel that transports chloride, bicarbonate, and other ions, and helps maintain epithelial tight-junctions and airway hydration. *CFTR* protein dysfunction results in altered epithelial transmembrane flow with fluid and chloride abnormalities, leading to luminal surface liquid dehydration with highly viscous secretions. Consequent chronic inflammation, recurrent infections, and epithelial damage result in the classic phenotype of CF with high pulmonary and nutritional morbidity and mortality.[3] Currently 2114 mutations have been

identified in the *CFTR* gene including 719 CF-causing mutations.[4,5]

Historically, CF has been described among European populations, however, emerging data reveal gross underestimation of the true epidemiology of CF in many low-income and middle-income countries (LMICs), including India.[6–10] The reported prevalence of CF in India based on immigrants in the USA and UK is 1/10,000 to 1/40,000,[8] however, updated data obtained directly from relevant populations are needed. While F508del is the most common (>85%) causative mutation among European ancestries, the genotype of non-European populations varies significantly,[7] and is poorly understood. F508del remains the most common mutation globally, however with a lower allele frequency among non-European populations,[7,9] specifically 27% among people with CF of south Asian origin.[11–13] In two independent studies conducted in north India, the carrier frequency of F508del was estimated to be 1 in 238 in a cohort of 955 live births by cord blood sampling,[14] and the carrier frequency of *any* pathogenic *CFTR* mutation to be 4.5% among 200 randomly selected healthy adults.[15]

An understanding of indigenous mutation patterns has significant implications in the planning of diagnostic algorithms and newborn screening programs, providing access to highly effective modulator therapy (HEMT), and in the development of standardised healthcare infrastructure for CF. As a systematic study to understand the CF genetic profile or the disease-burden has never been conducted in India, we report for the first time, the genetic analysis of 120 people with CF from areas of India and Bangladesh followed at a referral hospital in south India. Mutation analysis, genotype-phenotype correlations, novel mutations identified, regional variances, and clinical implications of these genetic data are described.

Methods

Study design

This is a retrospective descriptive analysis of data from people with CF followed at the paediatric respiratory clinic at Christian Medical College (CMC), Vellore in south India from September 2010 to August 2022. The team at the study site included a paediatric specialist, geneticists, biostatistician, and the CF-care team. The study was done in collaboration with the CF team at Nationwide Children's Hospital, Columbus, OH, USA. The Institutional review board at CMC, Vellore approved the data collection, and analysis of information (IRB Min No 14013(Observe) dated May 30, 2021).

The specific aims of this retrospective study were to (i) describe *CFTR* mutations of 120 people with CF followed at this single centre in south India, (ii) report any novel *CFTR* mutations identified in this cohort, (iii) describe genotype and selective phenotype features of the most common mutations identified, and (iv) highlight regional variances in genotype and phenotype among people with CF from south India and the Bengal-Bangladesh areas.

Clinical data collection

Diagnosis of CF

At this paediatric centre, 155 people with CF are or were followed during the study period. Diagnosis of CF was confirmed by either sweat chloride levels ≥60 mmol/L using pilocarpine iontophoresis or by identification of two known mutations by *CFTR* gene sequencing with deletion or duplication analysis on all people with CF (regardless of sweat chloride levels). Sweat conductivity testing had been done prior to availability of pilocarpine iontophoresis, and those with elevated levels (>80 mmol/L) had diagnostic confirmation by mutation analysis. One-hundred-twenty people with CF who had complete *CFTR* data, with two identified mutations were included in the study, and 35 people with CF (diagnosed by sweat testing) who were lacking mutation data either due to loss to follow up or early death were excluded. As this institution is a referral centre in the Indian subcontinent, this cohort included people with CF from four south Indian states, from east/northeast India, and the adjacent nation of Bangladesh (Fig. 1).

Clinical data collection

Retrospective review of de-identified information related to demographic characteristics, parental consanguinity, clinical parameters, sweat chloride levels, and *CFTR* mutation results were reviewed and analysed for the 120 people with CF included in the study. Limited longitudinal data regarding survival, spirometry (FEV_1% predicted), and BMI obtained during this study period are included for a small subset of this cohort.

CFTR mutation detection, categorisation, and analysis

Of the 120 samples included in the study, mutation analysis was completed in 90 samples at our institution by Next-Generation Sequencing[16] (NGS) using previously validated and internally developed protocols for targeted *CFTR* gene sequencing of extracted DNA. In the remaining 30 people with CF, diagnosis was confirmed by clinical exome sequencing at external laboratories in India. Details of NGS with library preparation, sequencing output, mutation analysis, annotation, and categorisation are documented in Supplementary Information. The CFTR primer pool included detection of intronic variants and deletion. When indicated, these results were validated by Sanger sequencing in the proband and the parents. All novel variants were classified per the American College of Medical Genetics and Genomics (ACMG) guidelines.[17] This is based on population frequency, in silico predictions, previous literature or databases describing the functional impact of the variant. Allele frequencies of known variants were compared with

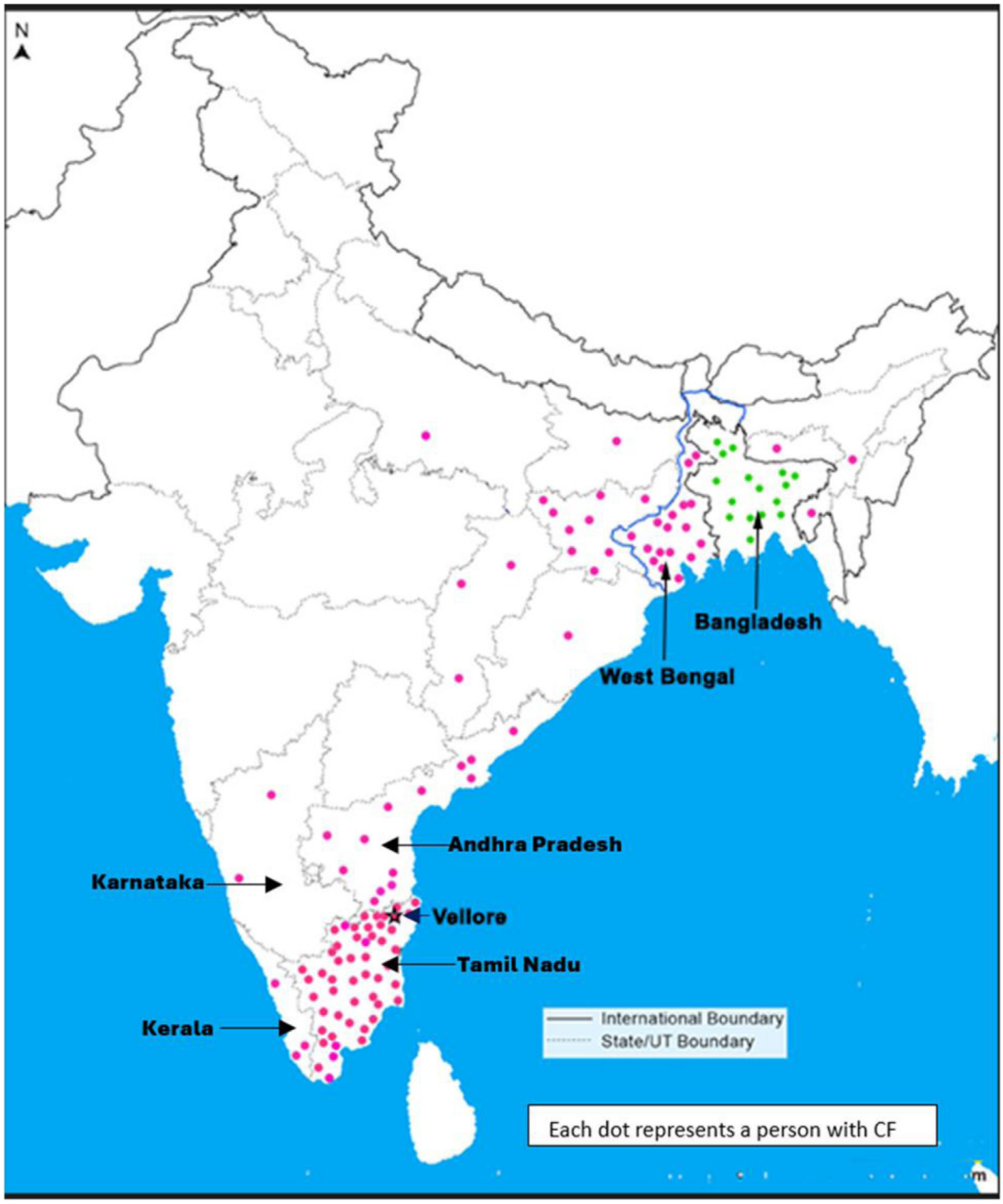

Fig. 1: Map of India showing residence locations of the people with cystic fibrosis (CF) included in the study. Each pink or green dot represents a person with CF.

two locus-specific databases from North America (*CFTR2*)[5] and France (CFTR-France).[18] External validation was restricted to genetic laboratories within India due to strict regulations on transfer of biological specimens. Clinical exome sequencing was done in laboratories accredited by the College of American Pathologists,[19] and Sanger validation was done in 23 cases (19%) which confirmed our laboratory's results.

Statistical analysis

Continuous variables were expressed as mean and SD if normally distributed, or median (IQR) if not normally distributed, and categorical data were expressed as number and percentages. The chi-square and Fisher's exact test were used to describe the association between categorical variables. All tests were two-sided at alpha (α) = 0.05 level of statistical significance. All the

statistical analyses were performed using Statistical Package for Social sciences (SPSS) version 21.0.

Role of the funding source

Funding from the CF Foundation and CMC, Vellore, India, were utilised for salary support for some members of the CF care team, for data collection, and for processing of samples collected for diagnostic and clinical purposes. The funders of the study had no role in study design, data analysis, data interpretation, or writing of the report.

Results

Demographic characteristics

Demographic data for the 120 included people with CF are described in Table 1. In this cohort, 104 people with CF were from India and 16 were from Bangladesh (Fig. 1). Among people with CF of Indian origin, 69 (66%) were from the four southern states [Tamil Nadu (where our institution is located), Kerala, Andhra Pradesh, and Karnataka] and 35 (34%) from eastern and north-eastern parts of India. This geographic distribution reflects the overall pattern of referrals received at this tertiary institution.[20]

Summarizing the demographic characteristics of this cohort of 120 people with CF from 113 families, 88 (73%) remain alive and 32 (27%) have subsequently died. Median age of the 88 people with CF (presumed alive), as of February 2023, was 9.79 years (range 1.16–22.66) with 55% being male. Median age at clinical diagnosis was 1.5 years (range 1 month–20 years) and 53% were diagnosed before 2 years of age (Table 1). Information on parental consanguinity was obtained from 112 (93%) of people with CF and in 83/112 (74%) of the people with CF, parents were non-consanguineous.

CFTR mutations identified in this cohort

Summary of identified mutations

Coverage analysis of NGS runs revealed an average base depth of 600× with 100% and 98% of the target having 20× and 98% with 100× coverage, respectively, achieving complete coverage of the target region ruling out any deletions in the gene. Clinical exome and in-house targeted panel output were validated and confirmed in 23 people with CF along with parental segregation analysis. In 240 alleles in 120 samples, 55 different variants were identified including 24 missense, ten nonsense and seven frameshift variants. Of these 55 variants, seven were at splice site regions, two were deep intronic, and two were deletions including one with a large deletion of exons 16 to 22. Fifty-five people with CF were homozygous, and 65 people with CF were compound heterozygous for the variants. The most common variant in the cohort was F508del (64/240 alleles with an allele frequency of 27%). F508del was identified in 46 people with CF including 18 people with CF with homozygous F508del, and 28 people with CF with compound heterozygous F508del, and 3849 + 10 kbC>T, c.1029delC, c.2052 dup, c.3484C>T and c.1367T>C. Table 2 describes allele frequency of 49 known *CFTR* variants identified in this cohort, and compares with the CFTR2 database.[5] Six novel variants are described separately in Table 3.

Novel variants

Six novel *CFTR* mutations were identified and submitted to ClinVar, National Library of Medicine[21] (submission ID: SUB12946844). These variants are reported in Table 3.

Position of variants on CFTR exons/intronic regions

CFTR variants were identified in 21 of 27 exons, with some exons emerging as hot spots for variants prevalent in the region. The most common hotspots were exon 11 (three individual variants distributed in 67/240 alleles), exon 14 (five variants in 28/240 alleles), exon 8 (three variants in 25/240 alleles) and exon 10 (two variants in 15/240 alleles) and intron 22 (two variants in 17/240 alleles). Fig. 2a and b describe the number of alleles with a variant at each exon or intron of the *CFTR* gene.

Genotype-phenotype correlations of the most common mutations identified in this cohort related to CFTR gene (among homozygous individuals)

The most common mutations in homozygous state were c.1521_1523delCTT, c.1802T>C, c.1029delC, c.2052 dup, c.3472C>T, c.2994 delA, c.1367T>C and c.3484C>T. Table 4 summarises the genotype and selective phenotype correlations of these mutations when identified in a homozygous state in at least two people with CF. Also included are limited longitudinal data for a subset of alive people with CF during this observation period with the most identified homozygous mutations: F508del and c.1029delC.

Regional distribution of variants

People with CF were predominantly from two geographically distinct regions: South India and the Bengal region (state of West Bengal, India, and the nation of Bangladesh, which was previously a province of India). Fig. 3 and Table 5 describe relative frequency of *CFTR* variants, including statistically significant differences in specific genotypes and phenotypes between these two regions.

Discussion

In summary, this is the first retrospective study to describe the *CFTR* mutation profile of people with CF from south India, and from certain regions of east and northeast India (Fig. 1). Our data augment awareness of the existence of CF in India, help define CF genetic

Current Age (in years), n = 88 (Living + lost to follow up/presumed alive)	
Median (IQR)	9.79 (5–15.5)
Age, n = 88	Number (%) of people with CF
<2 years	6 (6.8)
2 to <5 years	15 (17)
5 to <10 years	24 (27.3)
10 to <20 years	35 (39.8)
>20 years	8 (9.1)
Age at diagnosis (in years), n = 120 (living, lost to follow up, dead)	
Median (IQR)	1.5 (0.5–7.8)
Breakdown of age at diagnosis	Number (%) of people with CF
<2 years	63 (52.5)
2 to <5 years	15 (12.5)
5 to <10 years	17 (14.2)
10 to <20 years	24 (20.0)
>20 years	1 (0.8)
Diagnostic parameters	
Sweat chloride level available (n = 85)	Number (%) of people with CF
Diagnostic sweat chloride levels and 2 mutations	58 (48%)
Intermediate sweat with 2 mutations	22 (18%)
Normal sweat with 2 mutations	5 (4%)
Diagnosis based on two mutations without sweat chloride levels[b]	35 (30%)
Clinical parameters (mean [SD] unless otherwise stated)	
Nutrition:	
BMI at diagnosis for people with CF > 2 years old (n = 56) (Mean/SD)	14.5 kg/m^2 (3.35)
BMI Z-score at diagnosis	−2.96 (2.48)
For people with CF ≤ 2 years of age (n = 54)	
Weight-for-Length Z-score	−2.57 (2.01)
Weight-for-age Z-score	−4.57 (2.13)
Weight-for-age percentile	2.55 (8.81)
Pancreatic elastase assessment (n = 110)	
Pancreatic insufficient (faecal elastase < 200 mcg/gm of stool)	85 (77%)
Pancreatic sufficient (faecal elastase ≥ 200 mcg/gm of stool)	25 (23%)
Pulmonary:	
Spirometry data availability (n = 50) - Reporting first spirometry	
Mean FVC % pred (range)	70.3% (29%–109%)
Mean FEV_1% pred (range)	61.8% (18%–105%)
Mean FEF_{25-75}% pred (range)	41.7% (5%–123%)
Microbiology: Sputum culture availability (n = 108)	
Pseudomonas aeruginosa	78 (72%)
Methicillin-sensitive Staphylococcus aureus	33 (31%)
Methicillin- resistant Staphylococcus aureus	31 (29%)
Endocrinology: CF-related diabetes	6 (5%)
Survival	
Current living	80
Unknown due to loss to follow up (presumed alive)	8
Deceased	32
Mean age of death	5.2 years (1 month-24 years)
Death ≤ 2 years of age	18 (56%)
Death ≤ 6 months of age	14 (44%)
Most common mutations with high mortality rates, and % of people with CF with this mutation who died	c.1521_1523delCTT (33%) c.1029delC (12%)

Chest radiographs were available in all people with CF with varying pulmonary findings such as hyperinflation or recurrent pneumonias, but CT chest images were not available in most people with CF to report accurate data on bronchiectasis. [b]These people with CF either had sweat conductivity testing, or were acutely and terminally ill to undergo pilocarpine iontophoresis. Mutation analysis was completed with blood collected for clinical purposes.

Table 1: **Demographic characteristics of people with cystic fibrosis.**

Variant	Amino acid consequence	Allele frequency		Allele frequency CFTR2[a]		Variant determination
		N	Out of 240 alleles	N	Out of 142,036 alleles	
1. c.1521_1523delCTT	p. F508del	64	0.266	99,061	0.697,436	CF-causing
2. c.1029delC	p. Cys343Ter	21	0.08	21	0.000148	CF-causing
3. 3849+10kbC>T	intronic	15	0.06	1158	0.008153	CF-causing
4. c.1367T>C	p. Val456Ala	12	0.05	27	0.000190	CF-causing
5. c.2052 dup	p. Gln685ThrfsTer4	13	0.054	329	0.002316	CF-causing
6. c.3472C>T	p. Arg1158Ter	10	0.04	179	0.001260	CF-causing
7. C.1802T>C	p. Ile601Thr	8	0.033	0	0	ND
8. c.3484C>T	p. Arg1162Ter	8	0.033	651	0.004583	CF-causing
9. c.1393-1G>A	Splice variant	5	0.02	72	0.000507	CF-causing
10. c.2994 delA	p. Leu998PhefsTer2	5	0.02	0	0	ND
11. c.2T>C	p. Met1Thr	5	0.02	0	0	CF-causing[b]
12. c.2125C>T	p. Arg709Ter	4	0.016	63	0.000444	CF-causing
13. c.489G>A	p. Lys 163 = (splice variant)	4	0.016	0	0	ND
14. c.223C>T	p. Arg75Ter	3	0.012	92	0.000648	CF-causing
15. c.1000C>T	p. Arg334Trp	3	0.012	429	0.003020	CF-causing
16. c.1301C>G	p. Ser434Ter	3	0.012	16	0.000113	CF-causing
17. c.3944_3951del	p. Ile1315SerfsTer4	2	0.008	0	0	ND
18. c.274-1G>A	splice variant	2	0.008	40	0.000282	CF-causing
19. c.2805_2810delinsTCAGA	p. Pro936GlnfsTer6	2	0.008	1	0.000007	CF-causing
20. c.473G>A	p. Ser158Asn	2	0.008	0	0	ND
21. c.2290C>T	p. Arg764Ter	2	0.008	31	0.000218	CF-causing
22. c.2988+1G>T	Splice variant	2	0.008	0	0	ND
23. c.76A>G	p. Lys26Glu	2	0.008	0	0	ND
24. c.233 dup	p. Trp79LeufsTer32	2	0.008	11	0.000077	CF-causing
25. c.3717+40A>G	intronic	2	0.008	13	0.000092	CF-causing
26. c.3196 C>T	p. Arg1066Cys	2	0.008	220	0.001549	CF-causing
27. c.1117-1G>T	Splice variant	2	0.008	0	0	ND
28. c.1705T>G	P. Tyr569Asp	2	0.008	45	0.000317	CF-causing
29. c.4231C>T	p. Gln1411Ter	2	0.008	7	0.000049	CF-causing
30. c.709C>G	p. Gln237Glu	2	0.008	5	0.000035	Varying clinical consequence
31. c.253G>T	p. Gly85Ter	2	0.008	0	0	ND
32. c.2989-3C>G	Splice variant	1	0.004	0	0	ND
33. c.1646G>A	p. Ser549Asn	1	0.004	203	0.001429	CF-causing
34. c.38C>T	p. Ser13Phe	1	0.004	3	0.000021	CF-causing
35. c.3061C>A	p. Pro1021Ser	1	0.004	0	0	ND
36. c.1680A>T	p. Arg560Ser	1	0.004	0	0	ND
37. c.2834C>T	p. Ser945Leu	1	0.004	167	0.001176	CF-causing
38. c.3140–26A>G	Intronic	1	0.004	470	0.003309	CF-causing
39. c.80G>T	p. Gly27Val	1	0.004	0	0	ND
40. c.1505T>C	p. Ile502Thr	1	0.004	15	0.000106	CF-causing
41. c.4389 del	p. Ile1464LeufsTer4	1	0.004	0	0	ND
42. C.3874–4522A>G	Intronic	1	0.004	0	0	Varying clinical consequence[b]
43. c.1210-33_1210-6GT[12]T[4]	Intronic	1	0.004	182	0.001281	Varying clinical consequence
44. C.53+1G>T	Splice variant	1	0.004	8	0.000056	CF-causing
45. c.2374C>T	p. Arg792Ter	1	0.004	14	0.000099	CF-causing
46. c.2738A>G	p. Tyr913Cys	1	0.004	0	0	ND
47. c.349C>T	p. Arg117Cys	1	0.004	146	0.001028	CF-causing
48. c.1209G>T	p. Glu403Asp Splice variant	1	0.004	0	0	ND
49. Exon 16–21 deletion	Multi-exon	1	0.004	0	0	ND

Variant determination was based on Locus Specific DataBases (CFTR2) and CFTR-France (updated on September 22, 2023). ND: Not Determined. *CFTR* variants are not reported, and pathogenicity not determined in CFTR2 or any other locus-specific database. VUS: Variant of Unknown Significance. [a]https://cftr2.org/mutations (Website last updated on April 7, 2023[5]). [b]Variants 11 and 42 are not reported in CFTR2, but reported in CFTR-France https://cftr.iurc.montp.inserm.fr/cftr (Website last updated on Sept 22, 2023[18]).

Table 2: **Allele frequency of *CFTR* variants in this cohort and comparison with North American CFTR2 database (updated on August 16, 2023).**

Variant	Amino acid consequence	Exon	Number of alleles	Second mutation	Sweat test (mmol/L)	PI	Survival	ACMG[17] classification
1. c.1526G>T	p. Gly509Val	11	2	Homozygous	90	Yes	Died at age 7 years	Likely pathogenic
2. c.574G>T	p. Asp192Tyr	5	1	c.2374C>T	[a]Conductivity high >80 mmol/L	Yes	Advanced lung disease	Likely pathogenic
3. c.719T>G	p. Leu240Arg	6	1	c.2125C>T	78	No	Alive	Likely pathogenic
4. c.3119T>C	p. Leu1040Pro	19	1	c.1521_1523delCTT	81	Yes	Person with CF alive Two siblings died	Likely pathogenic
5. c.962delC[b]	p. Ser321LeufsTer7	8	1	c.1367T>C	51	No	Alive	Likely pathogenic
6. c.3434G>C	p. Trp1145Ser	21	2	Homozygous	74	Yes	Alive	Likely pathogenic

[a]Sweat chloride testing by Pilocarpine iontophoresis unless indicated; PI-Pancreatic insufficiency; ACMG: American College of Medical Genetics. PI = pancreatic insufficiency. [b]One person with CF, with variant c.962delC, had recurrent pneumonias, isolated *Pseudomonas*, had pseudo-Bartter syndrome, but was pancreatic sufficient and had intermediate sweat chloride levels.

Table 3: **List of novel variants in this cohort describing exon position, number of alleles with the variant and selected phenotypic features of each variant.**

profile of specific regions of India and Bangladesh, have diagnostic and clinical implications and, for the first time, demonstrate statistically significant regional variances in CF genotype and phenotype within India. We believe that these data will pique interest for further anthropologic studies and genetic profiling of CF as an autosomal recessive disease in a highly multi-ethnic and populous nation with high rates of consanguinity and migration.

The mutation profile in 120 people with CF with 240 alleles, included 55 different variants with a plethora of missense, nonsense, frameshift, and splice variants, along with deep intronic variants and large deletions. Fifty-five people with CF were homozygous, and 65 people with CF were compound heterozygous for the variants. We identified 49 known *CFTR* variants and six novel mutations with varying genotype-phenotype correlations, but most commonly with functional

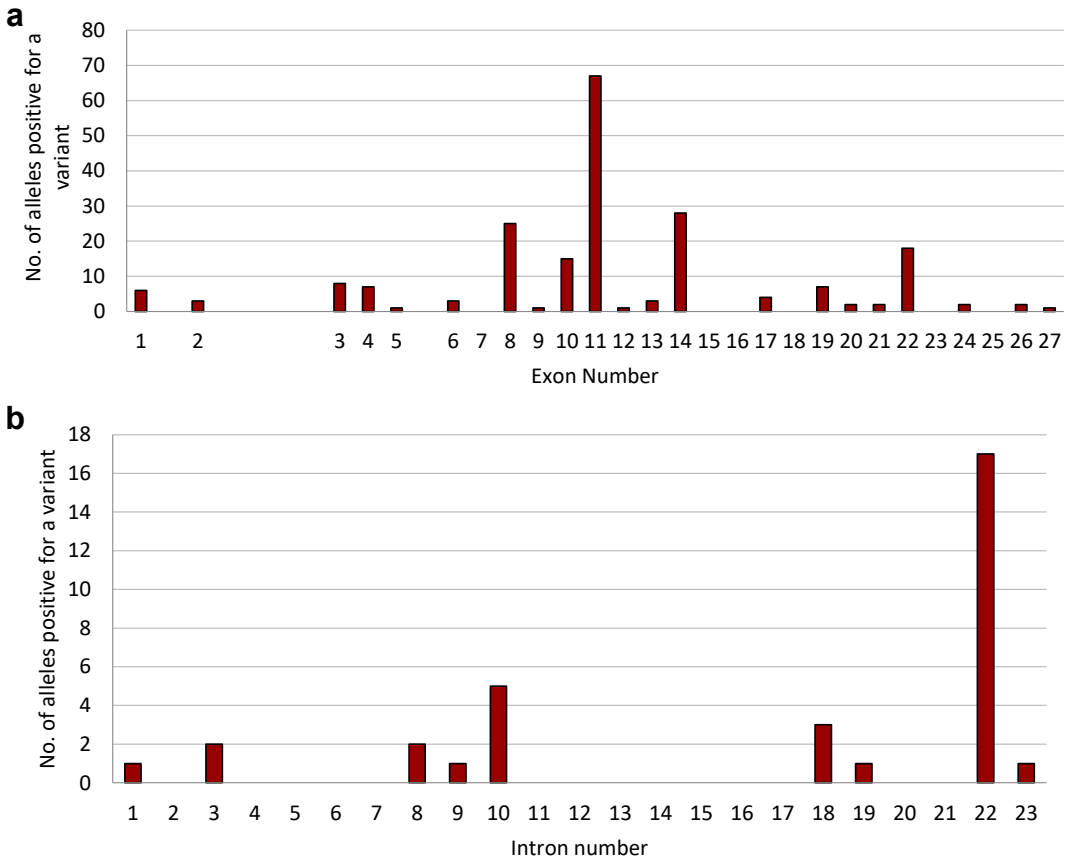

Fig. 2: a: Specific exons in the *CFTR* gene with number of alleles positive for a *CFTR* variant. b: Specific introns in the *CFTR* gene with number of alleles positive for a *CFTR* variant.

	c.1521_1523delCTT	c.1802T>C	c.1029delC	c.2052 dup	c.3472C>T	c.2994 delA	c.1367T>C	c.3484C>T
No. of people with CF who were homozygous	18	3	7	3	2	2	2	2
Mean current age (years)	9.83 (n = 9)	4.25 (n = 1)	4.6 (n = 4)	2.9 (n = 2)	5 (n = 1)	6.33 (n = 1)	18.25 (n = 1)	10.2 (n = 2)
Mean age at diagnosis (years)	0.85	1.1	1.32	0.13	0.25	0.38	10.62	0.5
Deceased (n)	9	2	3	1	1	1	1	0
Actively followed (n)	5	1	4	2	0	1	1	2
Status unknown (n)	4	0	0	0	1	0	0	0
Parental consanguinity in %	53 (n = 17)	33.3 (n = 3)	57 (n = 7)	66.6 (n = 3)	0 (n = 2)	50 (n = 2)	50 (n = 1)	0 (n = 2)
Number of siblings who died	6	0	1	0	2	0	0	1
Average sweat Chloride (mmol/L)	80.3 (n = 7)	81.5 (n = 2)	84 (n = 4)	92.5 (n = 2)	88.33 (n = 3)	72 (n = 2)	54 (n = 2)	Data n/a
Mean BMI at diagnosis (kg/m^2)	13.1 (n = 18)	13.4	12.4	12.46	13.44	10.8	15.2	13.3
Change in BMI at follow up (kg/m^2)	+0.3 (n = 6)[a]	Data n/a	−1.97 (n = 1)[b]	Data n/a	Data n/a	Data n/a	Data n/a	Data n/a
% of people with CF with low faecal elastase (<200 mcg/gm)	100 (n = 18)	100 (n = 3)	100 (n = 6)	100 (n = 3)	100 (n = 1)	100 (n = 2)	0 (n = 1)	100 (n = 1)
% of people with CF with PA isolation	66 (n = 15)	33.3 (n = 3)	60 (n = 5)	66 (n = 3)	100 (n = 1)	100 (n = 2)	100 (n = 2)	50 (n = 2)
Mean FEV_1%pred	53.4 (n = 4)	72.5 (n = 2)	53 (n = 1)	Data n/a	Data n/a	Data n/a	65.5 (n = 2)	48.5 (n = 2)
Change in mean FEV_1%pred at follow up[c]	−12.2 (n = 3)[c]	Data n/a	Data n/a	Data n/a	Data n/a	Data n/a	Data n/a	Data n/a

PA: *Pseudomonas aeruginosa*, Data n/a: Data not available. [a]Change in BMI over mean duration of 5.5 years for F508del. [b]Change in BMI over mean duration of 2 years for c.1028delC. [c]Change in FEV_1%pred at follow up at 3.3 years for F508del.

Table 4: **Genotype-phenotype correlations of the eight most common mutations in *CFTR* gene identified in this cohort (among homozygous individuals).**

consequences similar to the more severe class I to III mutations demonstrated elsewhere.[5] F508del was the predominant mutation overall but with an allele frequency of only 27% compared with 70% in North American databases.[5] Mutations with milder phenotype and pancreatic sufficiency (PS) were also noted, mainly from the Bengal cohort. The identification of six novel mutations was both interesting and alarming, as five were missense mutations, and the mutation c.962delC was a deletion variant that resulted in premature truncation of the *CFTR* protein. These novel mutations are probably 'pathogenic' or 'likely pathogenic' mutations per ACMG categorisation,[17] and have been submitted to ClinVar[21] for further clinical, historical, and functional validation. Hotspot exons and introns and targeted mutation panels are discussed below.

Overall, clinical manifestations of this cohort reflect the natural history of CF among unscreened populations.[1,22] This is a relatively young cohort with a median current age of people with CF of 10 years, with early onset of symptoms as reported in unscreened people with CF.[23] However, compared to prior reports from India, the trend of median age at diagnosis has reduced especially for those residing in south India (0.75 years vs. 3 years[7]), reflecting improved awareness of CF among physicians and families regarding early diagnosis and management. Clinical morbidity was high in this cohort. Moderate to severe protein-calorie malnutrition was prevalent in children aged >2 years (mean BMI Z-Score of −2.96 [SD 2.48] at time of diagnosis), consistent with prior reports,[24] and older children had moderate to severe obstructive defect (mean FEV_1% pred of 62%, range 18%–105%) with high rates of colonisation with *Pseudomonas aeruginosa* (72%), revealing a notable difference when compared with people with CF in North America where median BMI was 61st percentile for people with CF aged 2–19 years, median FEV_1%pred was 96% for people with CF aged 18 years, and *Pseudomonas* infection rates were 26%.[25] Interestingly, higher rates of PS were noted here compared to North American or European populations (23% versus 10–15%),[25,26] and the most common *CFTR* mutations associated with PS were c.3849+10kb and c.1367T>C, noted mainly among people with CF from Bengal and Bangladesh.

The death rate of 27% was alarming. Eighteen of 32 (56%) deaths were among children younger than two years, including 14 (44%) among infants younger than six months old. Our observation is probably just the tip of the iceberg of CF-related infant and childhood mortality in the Indian subcontinent,[27] and further epidemiologic assessments are needed urgently. Highly pathogenic *CFTR* variants F508del and c.1029delC were commonly observed in infant and early childhood deaths. The role of neonatal screening is irrefutable,[28,29] and many LMICs with suspected high incidence of CF will benefit tremendously with early screening to reduce preventable morbidity and mortality. In older children and adolescents, the sequelae of delayed diagnosis, unrecognised and inadequately treated CF co-morbidities, limited medical infrastructure and access to CF-specific medications, and socioeconomic factors contribute to premature death,[30] as also noted in our cohort. Here, the oldest living person with CF was 22

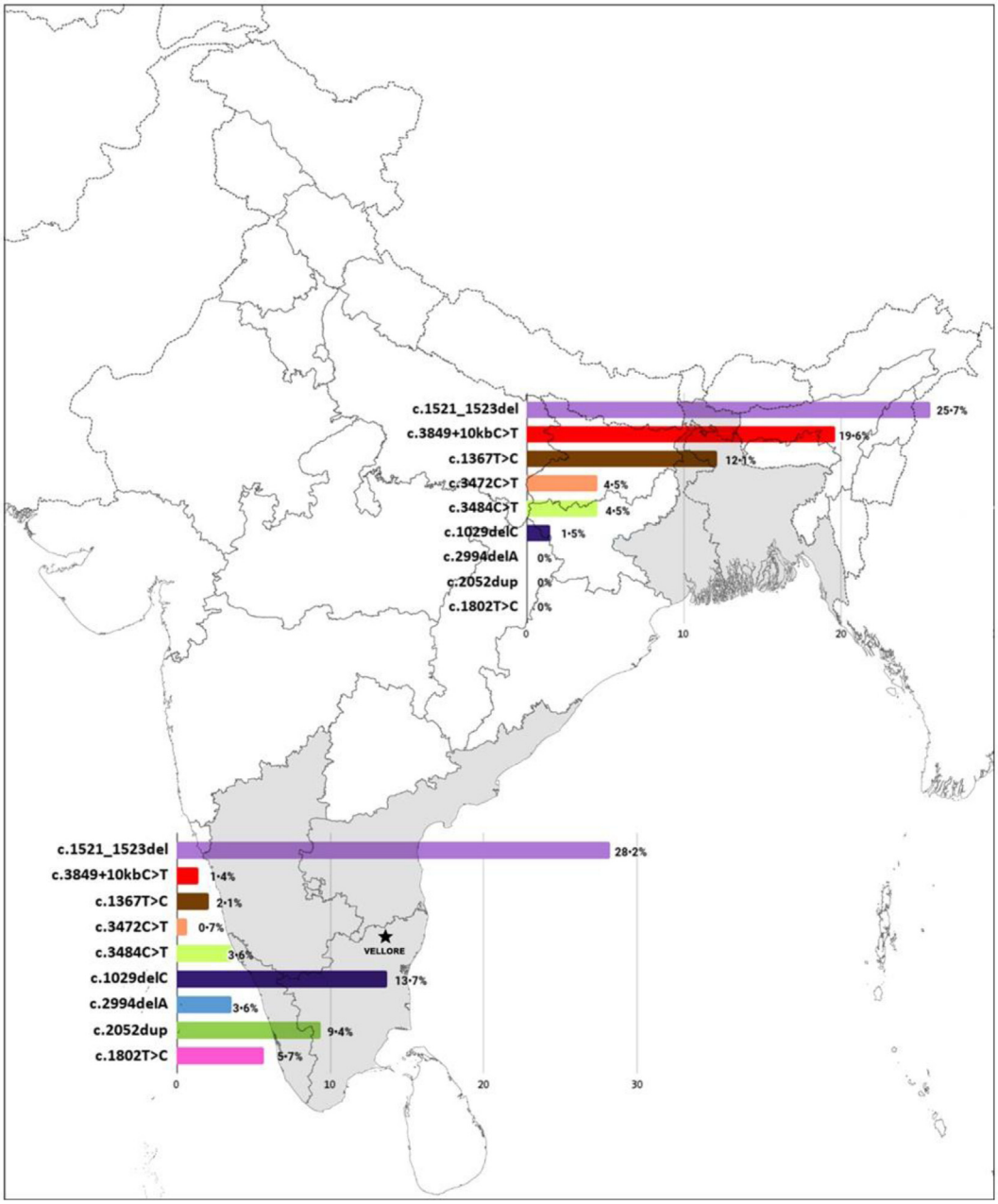

Fig. 3: Regional variances in *CFTR* mutation profile among people with cystic fibrosis from south India and the Bengal region (Indian state of West Bengal, and Bangladesh).

years, and the median age of death was 5.2 years. This is an astounding difference when compared to the USA's current median age of death of 33.9 years, with a predicted survival of 56 years.[31]

We identified significant differences in *CFTR* genetic profile and clinical manifestations based on the location of residence of people with CF. The Bengal region is more than 2000 km distant from the referral centre and findings of delayed age of diagnosis and higher median age of the Bengal cohort are likely due to non-representation of the younger or sicker individuals who could not have travelled across the country for treatment. While acknowledging the probable influence of selection and referral bias in the reported data, we highlight significant differences in the *CFTR* genotype of people with CF from south India versus the Bengal

Variables	Bengal region (N = 33)	Southern India (N = 69)	p-value
Gender, n (%)			
Male	13 (39.4)	41 (59.4)	0.058
Female	20 (60.6)	28 (40.6)	
Age at diagnosis n (%)			
<2 years	6 (18.2)	49 (71)	**<0.001**
≥2 years	27 (81.8)	20 (29)	
Median age at diagnosis in years (IQR)	7 (4.2–11.8)	0.75 (0.25–2.5)	**<0.0001**
Parental consanguinity, n (%)			
Present	0	25 (38.5)	**<0.0001**
Absent	30 (43)	40 (61.5)	
Sweat chloride mmol/l, n (%)			
Diagnostic (≥ 60 mmoL/l)	15 (57.7)	33 (78.6)	0.066
Intermediate (30–59 mmoL/l)	11 (42.3)	9 (21.4)	
Pancreatic function n (%)			
Pancreatic insufficiency	15 (50)	54 (85.7)	**<0.0001**
Pancreatic sufficiency	15 (50)	9 (14.3)	
Death, n (%)	7 (21%)	22 (32%)	0.264
Allele frequency	**Out of 66 alleles**	**Out of 138 alleles**	
Frequency of predominant variants n (%)			
c.1521_1523delCTT	17 (25.7)	39 (28.2)	0.71
3849+10kbC>T	13 (19.6)	2 (1.4)	**<0.00001**
c.1367T>C	8 (12.1)	3 (2.1)	**0.003**
c.3472C>T	3 (4.5)	1 (0.7)	0.06
c.3484C>T	3 (4.5)	5 (3.6)	0.75
c.1029delC	1 (1.5)	19 (13.7)	**0.006**
c.2994 delA	0	5 (3.6)	N/A
c.2052 dup	0	13 (9.4)	N/A
c.1802T>C	0	8 (5.7)	N/A

N/A: Not applicable, p-values < 0.05 were considered as statistically significant (in bold).

Table 5: **Comparison of phenotype between people with cystic fibrosis from the Bengal region (Indian state of West Bengal, and Bangladesh) and south Indian states (Tamil Nadu, Andhra Pradesh, Karnataka, and Kerala).**

region. West Bengal and the geographically adjacent country of Bangladesh were partitioned in 1947 and our observation confirms the genetic similarity of people with CF from this region. F508del was noted universally with an allele frequency of 27%, but our data suggest regional variances in some *CFTR* mutations across regions indicating ethnic correlations (Fig. 3 and Table 5). Clinically, people with CF from the Bengal region were more likely to be PS, have intermediate sweat chloride levels, and lower death rates compared to the south Indian cohort, although referral bias may have contributed to this observation. Regional differences or similarities in allele frequency of variants have been reported in other countries,[32,33] but not in India. In Jammu and Kashmir (one of India's northern-most Union Territories), a relatively higher frequency of intronic variant c.3849+10kb has been reported,[34] and interestingly, this variant was rarely noted in south India. However, mutations reported from other north Indian cohorts including c.1161delC and p. S549N[13] were not observed in our cohort. A comprehensive literature review of *CFTR* mutations from a few nations in Asia, including India by Singh and colleagues[35] identified 160 CFTR variants in approximately 3700 Asian CF chromosomes, including 11 relatively common, (specifically F508del, c.3849+10kb, p.S549N), and 24 rarer variants from north India (680 chromosomes). Similarly, 14 pathogenic *CFTR* mutations shared among people with CF from South Asia, including India (F508del, 1525-1G > A, G542X, S549N, R117H and others) were reported.[36] Overall, F508del remains the most common variant in Asia, albeit an allele frequency of 12–31%.[35] Further anthropologic studies are necessary to identify founder mutations and discern the role of ethnic admixture and migration patterns to understand these regional variations.

We explored the potential role of targeted mutation panels to enable population-specific and cost-effective diagnosis of CF applicable to our study regions. In 2001, CF became the first target of pan-ethnic universal

carrier screening by molecular methods. The ACMG[17] recommends a minimal panel of 100 disease-causing variants to be included in universal screening for those with no family history of CF, using targeted methods.[37] In Italy, detection rates of 95% and 95.6% were noted in two large-scale reports of people with CF from central and northern Italy, respectively, validating the utility of targeted CFTR panels for diagnosis, neonatal and carrier screening, and in CF genetic counselling.[38] Commercially available targeted screening panels are, however, strongly biased toward detection of reproductive risk and CF genetics in persons of European descent, and often do not include severe variants reported in non-European ethnicities, suggesting systematic population biases.[39] Mutational heterogeneity in the Indian subcontinent makes development of an inclusive diagnostic panel quite a challenge. Based on the number of registered people with CF in the North American database and the allele frequency of F508del, testing for a single mutation F508del would yield a confirmatory diagnosis (i.e., homozygous F508del) in 38% of people with CF, and would help identify at least one copy of F508del in 73% of people with CF in the American cohort.[5] In contrary, in the Indian cohort, testing for F508del solely would yield a confirmatory diagnosis in only 18 of 120 (15%) of people with CF, and identify at least one copy of F508del in 38% of people with CF. Our data reiterate the importance of creating expanded mutation panels for CF diagnosis in India and avoiding testing for F508del mutation alone. As reported here, four specific exons and one intron harboured 50% of the variants in this cohort. In retrospect, a targeted panel with variants in exons 8, 10, 11, 14 and intron 22 would have been diagnostic in 60 of 120 (50%) people with CF, and would have identified at least one copy of a mutation in 92 of 120 (76%) people with CF. However, further systematic epidemiological studies and analyses are necessary to confirm generalisability of our observations before validating region-specific applicability, diagnostic yield, and cost-effectiveness of such panels for our population.

In a society where CF is considered a rare disease or even non-existent, there could be negative implications related to intermediate sweat chloride levels and inconclusive diagnosis. Despite intermediate or even normal sweat chloride levels in symptomatic people with CF, 27 (23%) people with CF were diagnosed based on comprehensive mutation analysis, specifically those with c.3849+10kb and c.1367T>C mutations. The implication of inconclusive diagnosis, especially in our setting, is that it precludes empiric treatment of CF due to socio-cultural barriers with consequent progression of disease due to intermittent or no treatment.

In screened populations, people with CF who had PS often have minimal decline in lung function over their lifetime.[40] In our study, 13 people with CF had PS with non-diagnostic sweat chloride levels. Pulmonary disease was not recognised until progression to severe bronchiectasis, and two of these people with CF unfortunately died due to untreated pulmonary complications while physicians still questioned the diagnosis. We reiterate the importance of empiric initiation of basic CF therapies based on clinical symptoms, and the need for CF genetic testing in those with unconfirmed diagnosis.

Access to currently available *CFTR* modulator therapies remains a major challenge to CF care. In this cohort, 64 people with CF (53%) had HEMT-eligible mutations,[41] including 55 (46%) people with CF who were eligible for Elexacaftor/Tezacaftor/Ivacaftor (ETI) and 20 people with CF (17%) who had Ivacaftor-responsive mutations. Of the 32 people with CF who died, 16 had HEMT-eligible mutations, and would have qualified for HEMT (by age criteria) at the time of death, if they had resided in countries where ETI was initially approved, available and funded.[42] Indian pharmaceuticals are anticipating tentative approval for Ivacaftor, which needs to be urgently initiated for people with CF with Ivacaftor-eligible mutations especially those with advanced disease, and impending death. It is often an ethical dilemma for clinicians and families when HEMT-eligible mutations are identified, yet the people with CF cannot access or afford these life-changing medications due to exorbitant costs and patent legalities. Collaborative ventures by the global CF community can help improve awareness, offer clinical and diagnostic expertise, share resources, and engage in perseverant advocacy to initiate and direct changes in health equity, especially for people with CF who are underserved across the world.[43] In this context there is an urgent need for accurate CF genetic testing and improved access to HEMT in India.

Despite reporting highly valuable data, the authors acknowledge that these data are limited by a relatively small cohort from a single institution. There could have been sampling bias based on the geographical regions of residence, exclusion of people with CF due to death or loss to follow up, various ethnic backgrounds, and pertaining to people with CF from Bengal, bias in relation to who were fit or wealthy enough to embark on the journey to south India for treatment. Our data will add to the knowledge repository on *CFTR* mutations and CF disease burden in India but cannot be generalised to the whole nation of India or to people with CF of non-European ancestry, nor be utilised for calculating prevalence due to sample size and aforementioned biases. We report genotype-phenotype differences across regions, while acknowledging that these may not truly reflect the respective regions. However, this early report may pave way for more systematic studies to investigate regional differences. Statistical analyses were limited to descriptive interpretations at this time. Further genetic profiling of all newly diagnosed people with CF in India is necessary but unfortunately limited by costs.

Future research directions include projects to explore and collectively address important diagnostic and clinical needs to improve CF care in LMIC. As India is an ancient nation with major migration patterns and ethnic admixture over many centuries, it would be fascinating to decipher and map the *CFTR* mutation profile of various regions of India, and understand the impact of bio-, linguistic, and socio-cultural anthropology in the observed mutational heterogeneity. As more people with CF are identified in the Indian subcontinent, further genetic analyses may provide clarity on founder mutations, and whether these mutations are innate (most probably) or due to European admixture during the colonial or classical periods. However, urgent goals are to (i) improve access and affordability to genetic testing, (ii) create targeted mutation panels that would be highly sensitive, specific, practical, cost-effective, and be of regional-relevance, (iii) highlight HEMT-eligible mutations, (iv) validate many variants of unclear significance (VUS), and (v) contribute to the global *CFTR* databases and understanding of the true CF epidemiology. Further, it would also be pertinent to create an Indian CF registry to document actual disease burden, to help improve the infrastructure for neonatal screening, and to provide basic CF care. As the Indian subcontinent is home to 20–25% of the world population,[44] we are concerned that the incidence and prevalence of people with CF in these countries may far exceed the currently registered people with CF in all high-income countries.[10]

In conclusion, our single-centre data from south India confirms that CF does exist in India and is caused by a plethora of common, rare, and novel *CFTR* mutations with varying genotype-phenotype correlations. These highly pathogenic mutations compounded with the challenges of limited awareness of CF, delayed diagnosis, and inadequate CF-specific healthcare infrastructure contribute to serious and unrecognised health care burden. Understanding the genotype of people with CF from the Indian subcontinent is crucial to reduce morbidity and mortality, to direct drug development, and to improve access to affordable and sustainable treatment.

Contributors

SDV: Conceptualisation, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualisation, writing (original draft and revisions).

GRP: Conceptualisation, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualisation, writing and editing (original draft and revisions).

RA: Data curation, formal analysis, investigation, methodology, resources, software, validation, visualisation, writing (original draft and revisions).

AC: Data curation, formal analysis, investigation, methodology, resources, software, validation, visualisation, writing (original draft and revisions).

SD: Data curation, formal analysis, investigation, methodology, resources, software, validation, visualisation, writing (original draft and revisions).

PM: Data curation, formal analysis, resources, software, validation, visualisation, writing (original draft and revisions).

JRN: Data curation, formal analysis, methodology, project administration, resources, software, validation, visualisation, writing (original draft, specifically biostatistics).

Data sharing statement

The data that support the findings of this study are available from the corresponding author, [GRP], upon reasonable request after publication. Please email Grace.Paul@nationwidechildrens.org to request information.

Editor note

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Declaration of interests

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lansea.2024.100434.

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