BMJ Open

Diagnostic yield of cystic fibrosis from a South Australian monocentric cohort: a retrospective study

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To cite: Markulić J, Fuller M. Diagnostic yield of cystic fibrosis from a South Australian monocentric cohort: a retrospective study. *BMJ Open* 2025;**15**:e092209. doi:10.1136/bmjopen-2024-092209

► Prepublication history for this paper is available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2024-092209).

Received 08 August 2024
Accepted 16 December 2024

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ABSTRACT

Objectives To determine the diagnostic yield of cystic fibrosis (CF) using a two-tiered genetic testing approach. Although newborn screening includes CF, this typically only covers a selection of common genetic variants, and with over 2000 reported in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, we hypothesised that patients will be missed and present clinically later in life.

Design A retrospective study over a 5-year period (January 2018–December 2022).

Setting A single pathology service in South Australia.

Participants A total of 1909 CF test referrals from patients with clinical suspicion indicated by respiratory and gastrointestinal manifestations, foetal echogenic bowel and male infertility and asymptomatic CF requests for reproductive carrier screening.

Primary and secondary outcome measures The number and type of CFTR gene variants detected in symptomatic and asymptomatic testing referrals.

Results A total of 25 patients were diagnosed with CF or CF-related disorders (2.5%) with gastrointestinal symptoms yielding the highest diagnostic rate of 4.4%. Additionally, a total of 79 carriers (4.1%) were identified uncovering a carrier frequency of 1 in 24, which is consistent with the 1 in 25 reported in the Caucasian population. CF was found to be causative of foetal echogenic bowel in 0.83% of cases.

Conclusions This study highlights the importance of considering CF in symptomatic patients, even in a nation with >99% of newborns screened for CF. Additionally, the identification of CF in this population supports the recommendation for CF genetic testing in reproductive healthcare.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ A retrospective study to determine the diagnostic yield of cystic fibrosis (CF) in a region with >99% uptake of CF newborn screening.
⇒ A two-tiered genetic testing approach was used enabling a fast and cost-effective first-tier screen covering 90% of cystic fibrosis transmembrane conductance regulator (CFTR) variants, followed by next-generation sequencing that covered 98%.
⇒ The cohort could be clearly categorised based on symptomatology allowing for attribution of CFTR variants to specific manifestations.
⇒ There are two major limitations: the cohort only captured 5 years of data and was undertaken at a single pathology provider.

INTRODUCTION

Cystic fibrosis (CF) is caused by pathogenic variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that encodes the CFTR.[1–3] The CFTR protein forms a channel across the cell membrane, and when phosphorylated by cAMP-dependent phosphokinases, it allows the migration of chloride and bicarbonate ions outside of the cell.[4] Residing on the apical surface of epithelial cells in the respiratory, gastrointestinal and reproductive tracts, as well as in sweat and salivary glands, the CFTR protein regulates the hydration and pH of liquid that lines these cells. CF is therefore a multiorgan disease, affecting the respiratory, gastrointestinal and reproductive organs, although respiratory failure is the main cause of mortality.[5] In the lungs, defective CFTR channel function results in dehydration of the airway surface liquid, restricting the ability of ciliated epithelial cells to move mucus through the airways. Congested mucus precipitates a cycle of infection and inflammation damaging airways and culminating in respiratory failure.[6 7] In the gastrointestinal system, mucus builds up in the intestine, obstructing the secretion of digestive enzymes causing inflammation of the pancreas and an increase in gut acidity due to the lack of neutralising bicarbonate. Moreover, this environment disrupts the delicate microbiome, allowing colonisation of foreign bacteria, causing infections.[8 9] The CFTR channel similarly regulates the hydration of the epithelial cells lining the reproductive tract. In male patients, failed development of reproductive organs is caused by mucus accumulation that clogs the vas deferens, causing congenital absence of the vas deferens (CAVD) (occurring bilaterally (CBAVD) and unilaterally) resulting in obstructive azoospermia.[10–14] Genetic variants in the CFTR gene have also

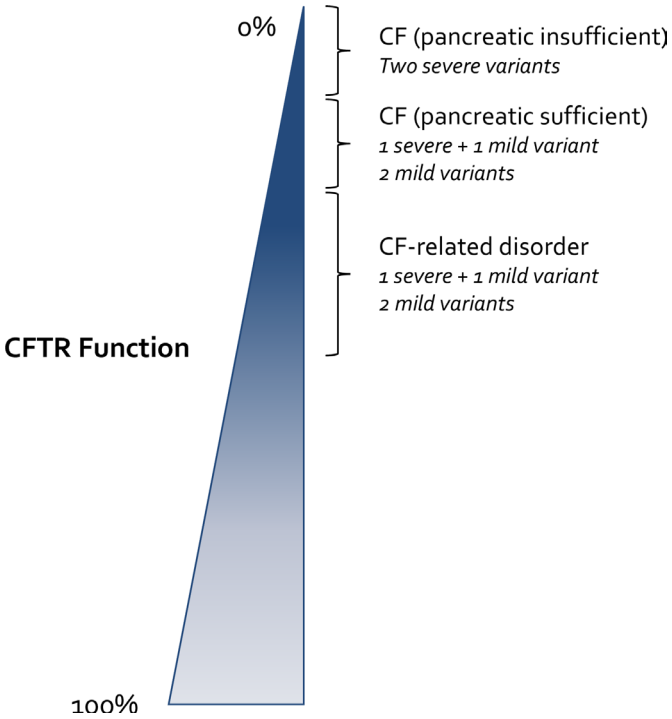

Figure 1 | Schematic diagram showing the spectrum of cystic fibrosis (CF) disorders corresponding to the level of cystic fibrosis transmembrane conductance regulator (CFTR) function. Variants causing a loss of or severely reduced CFTR channel function cause a severe form of CF (pancreatic insufficient). Variants with residual CFTR function cause a milder form of CF (pancreatic sufficient). Patients who do not meet the criteria for a CF diagnosis, but still contain variants in the CFTR gene, can be diagnosed with a CF-related disorder.

been found in other forms of male infertility including nonobstructive azoospermia and oligospermia.[15]

Since its discovery in 1989, over 2100 variants have been identified in the CFTR gene. However, not all variants have established clinical correlation. Since April 2023, 719 variants were listed as CF- causing in the CFTR2 database (CFTR.org), whereas 49 variants were described as having varying clinical consequences (VCC). From the previous database in April 2022, 319 variants were added, 318 of which were defined as CF-causing, although all are extremely rare with most detected in just one allele. Genotype–phenotype correlations are influenced by the functional impact of variants in *CFTR*; variants resulting in a complete loss of CFTR function typically associate with a severe phenotype and pancreatic insufficiency, whereas variants that allow residual function lead to milder phenotypes and are generally pancreatic sufficient (figure 1).[16 17] In severe CF disease, pancreatic insufficiency comprises 85% of all patients with CF.[18] The most common disease-causing variant in *CFTR* is c.1521_1523delCTT (commonly referred to as F508del), accounting for ~70% of alleles in the CFTR2 database. In Australia, 47% of patients diagnosed with CF are homozygous for c.1521_1523delCTT, while 43% are heterozygous (Australian Cystic Fibrosis Data Registry (ACFDR), Annual Report 2021). Just four alleles have >1% allele frequency in the CFTR2 database (excluding c.1521_1523delCTT), and the remaining are all <1%. The most common VCC variant is c.350G>A (commonly referred to as R117H), accounting for ~1.3% of alleles in the CFTR2 database. The c.350G>A variant is influenced by the splice acceptor site in intron 9, referred to as the CFTR poly-T and TG-repeats tract. The poly-T tract exists in three configurations: two efficient splice sites of 7T or 9T and one inefficient site of 5T; the presence of the 5T allele results in the absence of exon 10 in ~90% of *CFTR* mRNA expressed.[19] The adjacent TG-repeats region further influences the 5T; 12TG and 13TG repeats increase the penetrance of the 5T compared with 11TG repeats.[20 21] The 5T is therefore classified as a VCC variant by CFTR2 due to heterogenous clinical outcomes ranging from CF to CF-related disorder, when in combination with a CF-causing variant.[22 23]

The majority of CF cases are diagnosed neonatally in Australia with 76% made in the first 3 months of life (ACFDR, Annual Report 2021). This is largely due to the established newborn screening (NBS) programme, while, not mandatory, has a >99% participation rate.[24] About 12% of patients are diagnosed over the age of 18 years, and many adults diagnosed will have been born before the implementation of NBS (ACFDR, Annual Report 2021). Additionally, clinical presentation in adults is often phenotypically different from classical CF manifesting an attenuated phenotype with VCC or rare variants not covered in the *CFTR* newborn screen.[25 26] This also extends to CF-related disorders (CF-RD), a group of non-lethal diseases with variants in the CFTR gene, where patients do not meet the criteria for a CF diagnosis. There are three main CF-RD phenotypes all with CFTR dysfunction: CAVD, pancreatitis and bronchiectasis. Although they can be divided into two entities, CF and CF-RD form part of a continuous spectrum of disease associated with CFTR dysfunction; CF patients usually have two severe variants (classes I–III) or a severe and mild (classes IV–VI) variant, whereas CF-RD patients usually have two mild variants or a severe and mild variant (figure 1).[27]

In this study, we sought to determine the incidence of CF in postnatal patients with a clinical suspicion of CF referred to our laboratory for testing. This included patients with respiratory or gastrointestinal symptoms, male infertility and foetal echogenic bowel detected on ultrasound. The aim was to determine the diagnostic yield of CF in these cohorts to guide healthcare practice and genetic counselling in a nation with >99% uptake of CF NBS.

METHODS

This study compiled retrospective data on *CFTR* variant analysis from symptomatic and asymptomatic patient referrals to SA Pathology for the 5-year period from 1 January 2018 to 31 December 2022. A diagnosis of CF was

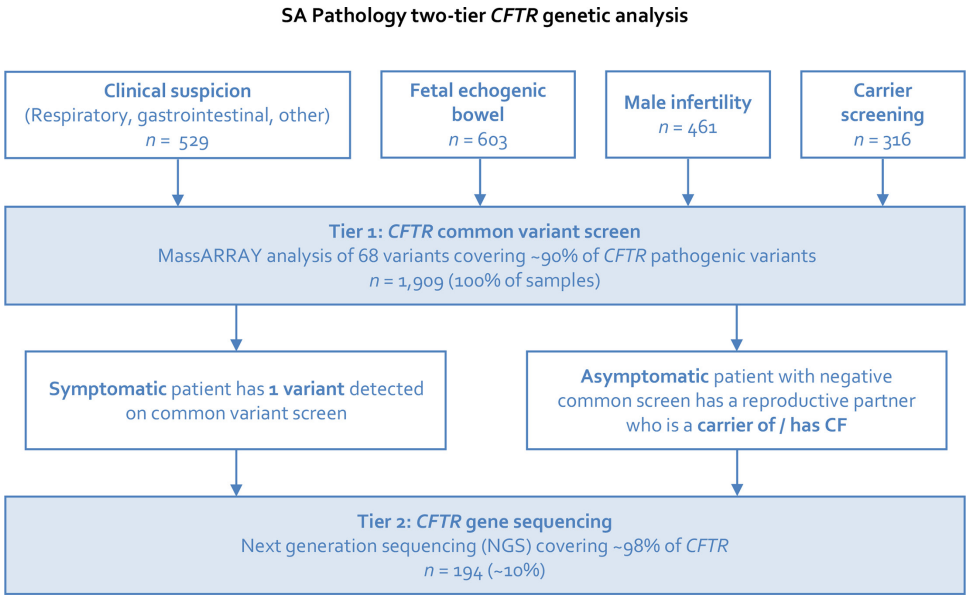

Figure 2 Two-tier screening process. In the study period from 1 January 2018 to 31 December 2022, 1909 samples were tested on the cystic fibrosis transmembrane conductance regulator (CFTR) common variant screen from symptomatic and asymptomatic cohorts. Approximately 10% of samples were reflexed to NGS. CF, cystic fibrosis.

made by the identification of two CF-causing variants in the CFTR gene, as dictated by the CFTR2 database.

A total of 1909 samples were included in this study. Symptomatic cohorts comprised clinical suspicion, foetal echogenic bowel (FEB) and male infertility. Asymptomatic cohorts included patients requesting reproductive carrier screening. The analysis did not include patients with a known family history of CF or NBS patients. In the 5-year period, SA Pathology employed a two-tier screening and diagnostic system for *CFTR* variants. The first tier involved a common variant screen that tested 68 *CFTR* variants using MassARRAY genotyping, covering ~90% of pathogenic CF variants, including those recommended by the American College of Medical Genetics and Genomics (ACMG).[28] A total of 1909 total samples were analysed using this method. If symptomatic patients had one variant detected or an asymptomatic patient with a negative common variant screen had a reproductive partner who was a carrier of, or had CF, samples were reflexed to next-generation sequencing (NGS). NGS covered all exons of the CFTR gene and copy number variation analysis. 194 samples were tested by NGS. A summary of the testing algorithm and sample numbers is shown in figure 2.

The diagnostic rate was determined by $f_d=n_p/n$, where n_p is the number of patients with two pathogenic *CFTR* variants and n is the total number of patients screened for that subgroup. Carrier frequency was determined by $f_c=n_p/n$, where n_p is the number of patients with one pathogenic *CFTR* variant and n is the total number of patients screened for carrier testing (asymptomatic). All statistical analyses were computed using GraphPad Prism (V9.0.0). Differences between study and population groups were established using the Binomial test, where p values<0.05 were considered significant. Annotation for p values used: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Patient and public involvement

Patients were not involved in the conception, conduct or interpretation of the findings.

RESULTS

Symptomatic testing

A total of 453 patients were tested with respiratory symptoms inclusive of chronic cough, bronchiectasis and recurrent respiratory infections and 22 patients (4.9%) had pathogenic *CFTR* variants detected (table 1). The most common pathogenic variant was c.1521_1523delCTT (32% of alleles detected), whereas 24 patients had the 5T polymorphism (46% of alleles detected) (figure 3). 9 patients (2%) were diagnosed with a CF or CF-RD (table 2), and 15 patients (3.3%) had one variant detected.

Of the 45 patients with gastrointestinal symptoms (pancreatitis, meconium ileus and bowel obstruction), seven (15.6%) returned pathogenic variants, all of which carried at least one c.1521_1523delCTT allele (table 1). Two (4.4%) patients were diagnosed with CF (table 2), and the remaining five patients (11.1%) were heterozygous for c.1521_1523delCTT.

Other clinical indications of CF included aquagenic wrinkling and test referrals where detailed clinical information was lacking. This included 31 patients, and one patient was diagnosed with CF (homozygous for c.1521_1523delCTT) and another heterozygous for c.1521_1523delCTT.

A total of 461 patients were tested with male infertility including presentations of CAVD, azoospermia and

Table 1 Summary of CF and CF-RD diagnoses and carriers in study subgroups

Presentation	Patients screened	Patients with variants detected (CF-causing, VCC and VUS)	Patients with CF-causing variants detected only	Total CF/CF-RD diagnoses (diagnostic frequency)	Total CF carriers† (carrier frequency)
Asymptomatic					
Carrier screening	316	25 (7.91%)	18 (5.70%)	N/A	18 (5.70%) **(0.15)**
Symptomatic					
Clinical suspicion: respiratory	453	24 (5.30%)	22 (4.86%)	9 (1.99%) **(<0.0001****)**	15 (3.31%) **(0.55)**
Clinical suspicion: gastrointestinal	45	7 (15.6%)	7 (15.6%)	2 (4.44%) **(0.0002***)**	5 (11.1%) **(0.033*)**
Clinical suspicion: other	31	2 (6.45%)	2 (6.45%)	1 (3.23%) **(0.0123*)**	1 (3.23%) **(>0.99)**
Male infertility	461	28 (6.07%)	28 (6.07%)	13 (2.82%) **(<0.0001****)**	16 (3.47%) **(0.48)**
Foetal echogenic bowel	603	29 (4.81%)	24 (3.98%)	N/A‡	24 (3.98%) **(>0.99)**
Total	1909	115 (6.02%)	101 (5.3%)	25 (2.5%)	79 (4.1%)

Values in non-bold brackets show diagnostic and carrier frequency calculated as a percentage of patients screened for that cohort. Values in bold brackets show statistical significance of diagnostic and carrier frequency compared with a population diagnostic frequency of 0.04% and population carrier frequency of 0.4%. Statistical significance was calculated using the Binomial test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). Exact p values are shown in brackets in bold.
†Total CF carriers calculated with patients carrying CF-causing variants only.
‡Three couples were both found to be carriers; prenatal testing found all three foetuses diagnosed with CF.
CF, cystic fibrosis; CF-RD, cystic fibrosis-related disorder; VCC, varying clinical consequences; VUS, variant of uncertain significance.

oligospermia, and 28 patients (6.1%) had pathogenic *CFTR* variants (table 2). The most common pathogenic variant was c.1521_1523delCTT (26% alleles), while 43 patients harboured the 5T polymorphism (56% alleles) (figure 3). From the total number of patients tested, 13 (2.8%) were diagnosed with a CF or CF-RD, including seven patients with the 5T polymorphism in combination with a CF-causing variant, resulting in a CF-RD diagnosis (table 2). 16 patients (3.5%) carried one variant, and 35 patients (7.6%) carried the 5T polymorphism only.

Pregnant patients with FEB detected on ultrasound as well as the patient's partner, without a history of CF, were also tested. From 603 tests, encompassing 363 pregnancies (not all male partners were referred for testing), 24 patients (4%) carried CF-causing variants (table 1). Again, the most common pathogenic variant was c.1521_1523delCTT (38% of alleles) (figure 3). Carriers of the 5T polymorphism (55% of alleles) were identified in 36 patients. If one patient of the reproductive couple was found to be a carrier of a CF-causing variant, then the partner was reflexed to NGS; VCC and variant of uncertain significance (VUS) variants were reported in five partners (excluding 5T).

Carrier screening

Carrier screening was conducted in 316 patients referred for reproductive carrier screening and patients whose partner was either a carrier of, or was diagnosed with, CF. Carriers of a CF variant were found in 25 patients, of which 18 carried a CF-causing variant, two carried VCC variants and five carried a VUS (table 1 and figure 3). The carrier frequency of this group was determined by taking the number of patients with pathogenic variants detected (18), divided by the total number of patients requested for carrier screening (316), which was calculated to be 1 in 18 (5.7%).

DISCUSSION

Studies of CF diagnoses in neonates are well established with the near global implementation of neonatal screening programmes; however, there are limited contemporary investigations on CF diagnoses in symptomatic individuals. We aimed to fill this gap by determining the diagnostic and carrier frequency of CF in symptomatic cohorts in a retrospective study spanning the last 5 years. The highest diagnostic frequency was observed in patients with gastrointestinal symptoms (table 2), and this cohort showed the highest frequency of c.1521_1523delCTT. The most common gastrointestinal manifestation noted in this study was recurrent pancreatitis, a CF-RD. A study by Bishop *et al* analysed 56 patients presenting with pancreatitis and found that 43% carried one *CFTR* variant and 11% carried two *CFTR* variants, noting that the diagnostic criteria for CF could be fulfilled

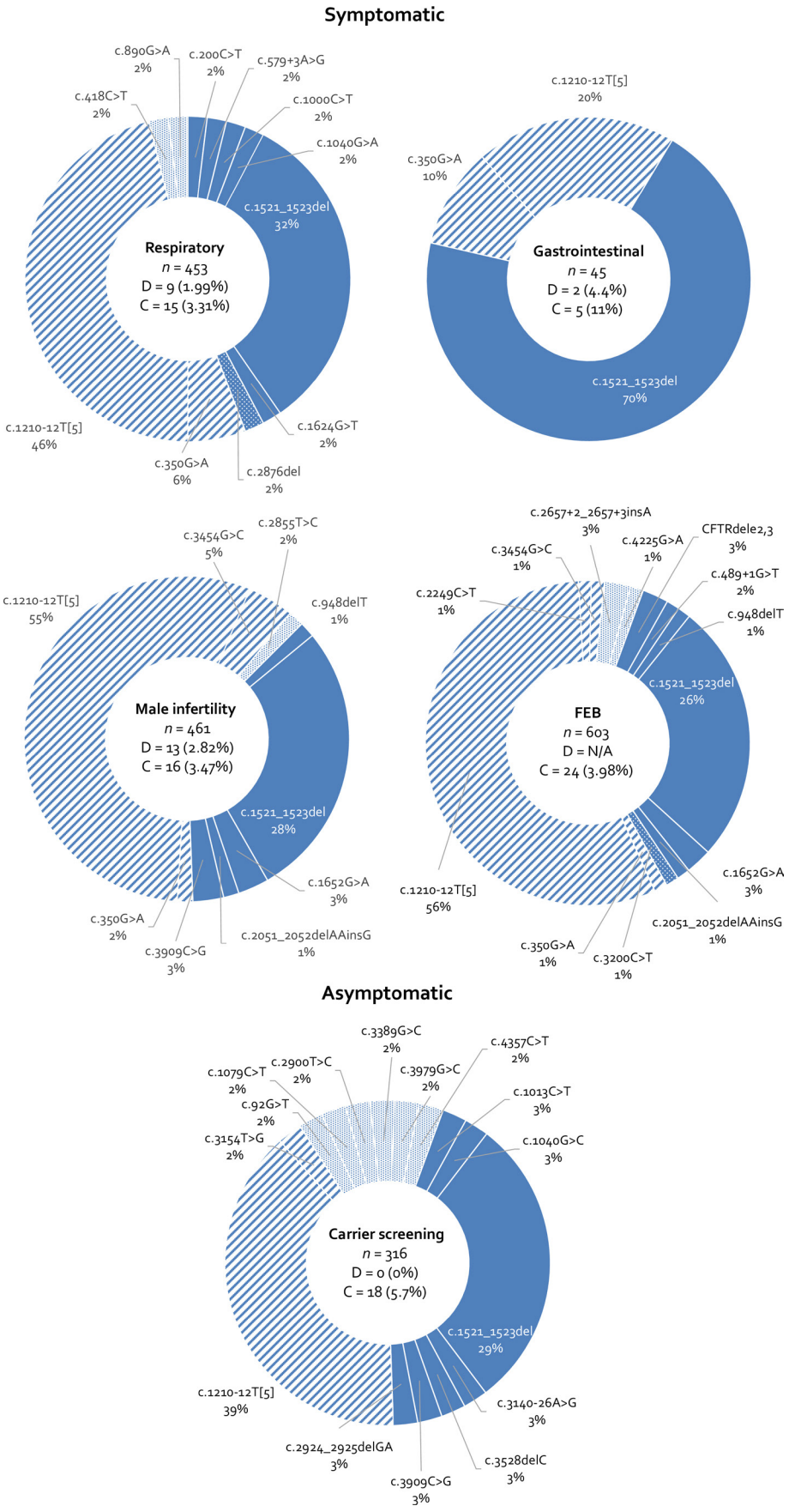

Figure 3 CFTR variants identified in study subgroups. Variants were classified as per CFTR2 database: CF-causing (solid), varying clinical consequences (stripes) and variant of uncertain significance (dark spots white background). Novel likely pathogenic variants (white spots grey background) were classified as per ACMG Guidelines. FEB, foetal echogenic bowel.

Table 2 Reported cystic fibrosis and CF-RD variant combinations in study subgroups

Presentation	Variant combination	Alleles (n)
Clinical suspicion: respiratory	c.1521_1523delCTT (p.Phe508del)/c.1521_1523delCTT (p.Phe508del)	3
	c.350G>A (p.Arg117His);T5/c.1521_1523delCTT (p.Phe508del);T9	1
	c.1000C>T (p.Arg334Trp)/c.1000C>T (p.Arg334Trp)	1
	c.1521_1523delCTT (p.Phe508del);T9/T5	1
	c.1521_1523delCTT (p.Phe508del)/c.579+3A>G	1
	c.1521_1523delCTT (p.Phe508del)/c.1040G>A (p.Arg347His)	1
	c.1521_1523delCTT (p.Phe508del)/c.2876del (p.Ala959Aspfs*9)	1
Clinical suspicion: gastrointestinal	c.350G>A (p.Arg117His);T7/c.1521_1523delCTT (p.Phe508del);T9	1
	c.1521_1523delCTT (p.Phe508del)/c.1521_1523delCTT (p.Phe508del)	1
Clinical suspicion: other	c.1521_1523delCTT (p.Phe508del)/c.1521_1523delCTT (p.Phe508del)	1
Male infertility	c.1521_1523delCTT (p.Phe508del);T9/T5	4
	c.54–5940_273+10 250del21kb (CFTRdele2,3), T5/T7*	2
	c.350G>A (p.Arg117His), T5/T7*	1
	c.489+1G>T(p.?)/c.2657+2_2657+3 insA (p.?), T7/T9*	1
	c.1521_1523delCTT (p.Phe508del);T9/c.3200C>T (p.Ala1067Val);T7	1
	c.1521_1523delCTT (p.Phe508del);T9/c.2249C>T (p.Pro750Leu);T7	1
	c.1521_1523delCTT (p.Phe508del);T9/c.4225G>A (p.Glu1409Lys);T9	1
	c.1652G>A (p.Gly551Asp);T7/c.2657+2_2657+3 insA (p.?);T7	1
	c.2051_2052delAAinsG (p.Lys684SerfsX38), T5/T7*	1

*Phasing not determined.
CF-RD, cystic fibrosis-related disorder.

in 21% of these patients.[29] A high carrier frequency was also observed, in which five patients (11%) were found to be heterozygous, significantly higher than the population carrier frequency of 4% (table 1). However, the sample size of this cohort (45 patients) was a fraction of the respiratory presentation cohort (453 patients).

Respiratory manifestations, including chronic wet cough, bronchiectasis and frequent respiratory tract infections, covered the other major symptomatic cohort. Here, we identified nine patients (2%) with two variants, and 15 patients (3.3%) with one variant, out of a total of 453 patients (table 1). Almost half of the patients had the 5T polymorphism (table 3), and one patient had the 5T in trans with the c.1521_1523delCTT variant.

Male patients presenting with infertility were one of the largest cohorts in this study. CBAVD is found in 95% of male patients with CF, and *CFTR* variants are found in 78% of male patients with CBAVD, 46% with CU(unilateral)AVD and up to 18% with oligospermia.[15] In 30%–45% of male patients with CBAVD, the attributable genotype is typically CF_{severe}/CF_{mild}, CF_{mild}/CF_{mild}, CF_{severe}/5T and CF_{mild}/5T.[30] The patients in this study presented with oligospermia, azoospermia and CBAVD; a total of 461 patients were tested and 12 male patients (2.8%) met the criteria for a CF-RD diagnosis (table 2). The most common genotype was c.1521_1523delCTT in trans with the 5T polymorphism found in four patients consistent with a previous report of this being the most common variant combination observed for male patients with CBAVD.[31] The large deletion variant, c.54–5940_273+10 250del21kb (known as CFTRdele2,3 in legacy nomenclature), in trans with 5T was detected in two patients, and large genomic rearrangements have been reported in trans with 5T in male patients with CBAVD.[32] The rare c.2657+2_2657+3 insA variant—currently under evaluation by the CFTR2 database—was detected in two patients. This variant has been reported in patients with CF-RD, including obstructive azoospermia in male patients.[33] The c.2249C>T is a VCC according to the CFTR2 database, and although c.3200C>T and c.4225G>A are not in CFTR2, c.3200C>T has been reported in male patients with CBAVD, in trans with c.1521_1523delCTT, as is the case here.[34] 16 patients were found to be carriers of a pathogenic *CFTR* variant, supporting previous studies and reporting that pathogenic *CFTR* variants are higher in male patients with fertility issues compared with fertile male patients. The systematic review into CBAVD patients by Yu *et al* found that 78% of patients had at least one variant, 46% had two variants and 28% had only one variant.[35] Additionally, more than half (56%) of this cohort had the 5T polymorphism (table 3) in agreement with Yu *et al* reporting the 5T polymorphism as the most common detected in ~25% of CBAVD patients.

The FEB group, which included pregnant patients and partners (with no history of CF), was the largest in this study with 603 patients screened, covering

Table 3 Cystic fibrosis transmembrane conductance regulator (CFTR) variants identified in this study

Variant cDNA name	Variant protein name	Variant legacy name	Variant classification as per CFTR2 database	Alleles	Allele frequency (of 247 alleles detected) (%)	Allele frequency (of 3818 chromosomes) (%)
c.1210-12T(5)	p.?	5T	Varying clinical consequence	121	49.0	3.17
c.1521_1523delCTT	p.Phe508del	F508del	CF-causing	76	30.8	1.99
c.350G>A	p.Arg117His	R117H	Varying clinical consequence	6	2.43	0.157
c.1652G>A	p.Gly551Asp	G551D	CF-causing	4	1.62	0.105
c.3454G>C	p.Asp1152His	D1152H	Varying clinical consequence	4	1.62	0.105
c.3909C>G	p.Asn1303Lys	N1303K	CF-causing	3	1.21	0.0786
c.54–5940_273+10250del	p.Ser18ArgfsX16	CFTRdele2,3	CF-causing	2	0.810	0.0524
c.948delT	p.Phe316LeufsX12	1078delT	CF-causing	2	0.810	0.0524
c.2051_2052delAAinsG	p.Lys684SerfsX38	2183AA->G	CF-causing	2	0.810	0.0524
c.2657+2_2657+3insA	p.?	2789+2insA	Unknown significance	2	0.810	0.0524
c.92G>T	p.Arg31Leu	R31L	Unknown significance	1	0.405	0.0262
c.200C>T	p.Pro67Leu	P67L	Unknown significance	1	0.405	0.0262
c.418C>T	p.Pro140Ser	P140S		1	0.405	0.0262
c.489+1G>T	p.?	621+1G->T	CF-causing	1	0.405	0.0262
c.579+3A>G	p.?	711+3A->G	CF-causing	1	0.405	0.0262
c.890G>A	p.Arg297Gln	R297Q		1	0.405	0.0262
c.1000C>T	p.Arg334Trp	R334W	CF-causing	1	0.405	0.0262
c.1013C>T	p.Thr338Ile	T338I	CF-causing	1	0.405	0.0262
c.1040G>A	p.Arg347His	R347H	CF-causing	1	0.405	0.0262
c.1040G>C	p.Arg347Pro	R347P	CF-causing	1	0.405	0.0262
c.1079C>T	p.Thr360Ile	T360I		1	0.405	0.0262
c.1624G>T	p.Gly542X	G542X	CF-causing	1	0.405	0.0262
c.2249C>T	p.Pro750Leu	P750L	Varying clinical consequence	1	0.405	0.0262
c.2855T>C	p.Met952Thr	M952T	Unknown significance	1	0.405	0.0262
c.2876delC	p.Ala959Aspfs*9*			1	0.405	0.0262
c.2900T>C	p.Leu967Ser	L967S	Varying clinical consequence	1	0.405	0.0262
c.2924_2925delGA	p.Arg975IlefsX10	3056delGA	CF-causing	1	0.405	0.0262
c.3140–26A>G	p.?	3272–26A->G	CF-causing	1	0.405	0.0262
c.3154T>G	p.Phe1052Val	F1052V	Varying clinical consequence	1	0.405	0.0262
c.3200C>T	p.Ala1067Val	A1067V		1	0.405	0.0262
c.3389G>C	p.Gly1130Ala	G1130A		1	0.405	0.0262
c.3528delC	p.Lys1177SerfsX15	3659delC	CF-causing	1	0.405	0.0262
c.3979G>C	p.Val1327Leu	V1327L		1	0.405	0.0262
c.4225G>A	p.Glu1409Lys	E1409K		1	0.405	0.0262

Continued

Table 3 Continued

Variant cDNA name	Variant protein name	Variant legacy name	Variant classification as per CFTR2 database	Alleles	Allele frequency (of 247 alleles detected) (%)	Allele frequency (of 3818 chromosomes) (%)
c.4357C>T	p.Arg1453Trp	R1453W		1	0.405	0.0262
Total				247		

Allele frequency and classification of CFTR variants detected in this study. Variants not included in the CFTR2 database have classification omitted.
*The c.2924_2925delGA variant was added to the database in 2023 and has now been classified as CF-causing; at the time of reporting, this variant was classified as a variant of uncertain significance.

363 pregnancies. Over the study period, 24 patients (4%) were found to be carriers of CF (table 1). Three couples had prenatal testing, revealing positive CF diagnoses in the foetus (c.948delT / c.1521_1523delCTT; c.1521_1523delCTT/c.3454G>C; c.1521_1523delCTT/c.2051_2052delAAinsG). Therefore, this study proposes CF to be the cause of FEB in 0.83% of cases (3 of 363 pregnancies), supporting the need to offer testing to patients and their partners when FEB is detected. One partner had a VUS detected on NGS, c.2855T>C, which is currently under evaluation by CFTR2 (table 3). The FEB group also had a high detection rate for 5T, with over half (55%) of patients screened carrying this polymorphism.

This study also investigated asymptomatic patients with no history of CF to ascertain carrier frequency by way of including general test requests for reproductive screening, as well as partners of CF carriers and those diagnosed with CF. Approximately half (114 patients) of this cohort comprised patients who had a partner with CF or were a CF-carrier. As part of the two-tier screening process for partners of CF carriers/diagnosed, 103 individuals were reflexed to NGS in search for rarer variants and 10 had variants detected, 3 of which were CF-causing (c.1013C>T, c.2924_2925delGA and c.3140–26A>G), 2 VCC (c.2900T>Cand c.3154T>G) and 5 VUS (c.92G>T, c.1079C>T, c.3389G>C, c.3979G>Cand c.4357C>T) variants. Therefore, excluding VCC and VUS heterozygous patients, we found that 12.3% (14 of 114) of partners were identified as carriers of CF, significantly higher (***p=0.0002) than the general population carrier frequency. Individuals requesting general carrier screening only four (1.9%) had CF-causing variants, two were heterozygous for the c.1521_1523delCTT variant, one for c.1040G>Cand one for c.3909C>G. The systematic review by Ioannou *et al* found 61–100% of partners of carriers sought carrier testing for CF.[36] This is due to knowledge of CF and access to genetic counselling, both important factors in the uptake of CF testing.

Overall, 34 variants were detected (table 3) with c.1521_1523delCTT the most common, comprising ~30% of all variants detected. Variation in c.1521_1523delCTT allele frequency is observed across Europe, where it is found at 75.3% and 87.5% in the UK and Denmark, respectively, and at 53% and 24.5% in Greece and Turkey, respectively.[37] While in Africa, the allele frequency of c.1521_1523delCTT is significantly lower at 20% and 17.6% in Algeria and Tunisia, respectively.[37] The allele frequency of c.1521_1523delCTT in Asia is 12–31%.[38] While CF is common in Caucasian populations, at an incidence of approximately 1 in 2,500, population-specific carrier frequencies, variants and CF manifestations are well established.[39] In Australia, while the most common ancestry is English, other common ancestries include Chinese, Indian, Scottish, Irish and Italian, and approximately 17% of the Australian population identify with Asian ancestry (Australian Bureau of Statistics, Cultural Diversity 2021 Census). Therefore, the spectrum of variants identified could be attributed to the diverse Australian population.

The 5T polymorphism comprised almost half (49%) of all variants detected (table 3). Large cohort studies have determined the allelic frequency of 5T to be ~4%.[40] In this study, we determined the allelic frequency of 5T to be 3.2% (121 of 3818 chromosomes) (table 3). In fact, the allelic frequency of the 5T polymorphism is similar to the collective frequency of all the CF variants detected in our cohorts, at 3.3%. The 5T polymorphism was most prominent in the male infertility cohort, which also had the most diagnoses of 5T in trans with a CF-causing variant (table 2). The high prevalence of the 5T polymorphism is thought to be attributed to its milder spectrum of symptoms compared with severe *CFTR* variants, as is the case with other mild CF variants. In the CFTR2 database, the allele frequency of 5T is approximately 0.4%; however, this database is primarily for severe (classic) CF diagnoses and the 5T polymorphism is more commonly associated with mild CF and CF-RD. Although the 5T allele has variable penetrance based on the status of the adjacent TG-repeats, higher TG-repeats in cis with 5T typically increase penetrance and severity of 5T, compared with individuals with fewer TG-repeats in cis with 5T.[20 21] The common variant screen using MassARRAY only included poly-T detection (5T, 7T or 9T); the TG-repeats status was determined in a handful of cases when requested. However, in February

2023, the ACMG published updated guidelines for *CFTR* variant reporting, noting that the TG-repeats should be reported whenever 5T is detected, due to its variable penetrance.[41] In conclusion, the Australian population offers a unique landscape to monitor and collate contemporary epidemiological data that reflect a pan-ethnic population, improving healthcare policies and patient care. The results from this study support the notion that requests for *CFTR* genetic testing are appropriate and should be recommended to patients with clinical symptoms even in a nation with a highly compliant (>99%) NBS programme. A limitation of this study is noteworthy, as referrals included only those from the state's public pathology provider and therefore unable to capture all requests from the South Australian population.

Acknowledgements The authors would like to thank all the staff involved in the laboratory testing of cystic fibrosis in Genetics and Molecular Pathology at SA Pathology for their analytical work.

Contributors MF: conception, critical review and editing. JM: data compilation, data analysis and writing. MF: serves as the guarantor and accepts full responsibility for the work and/or the conduct of the study, had access to the data and controlled the decision to publish.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Women's and Children's Hospital Human Ethics Committee (2023/GEM00020). Patients consented to have the testing done and results are all blinded as reported and required by ethics.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as online supplemental information.

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