BMJ Open

The diagnostic yield of cystic fibrosis in symptomatic cohorts: a retrospective study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-092209
Article Type:	Original research
Date Submitted by the Author:	08-Aug-2024
Complete List of Authors:	Markulic, Jasmina; SA Pathology, Genetics and Molecular Pathology Fuller, Maria; SA Pathology, Genetics and Molecular Pathology; The University of Adelaide Faculty of Health and Medical Sciences,
Keywords:	Cystic fibrosis < THORACIC MEDICINE, Prevalence, GENETICS

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

The diagnostic yield of cystic fibrosis in symptomatic cohorts: a retrospective study

Jasmina Markulic PhD [1,2] and Maria Fuller PhD [1,2,3*]

[1]Genetics and Molecular Pathology, SA Pathology at Women's and Children's Hospital, North Adelaide, South Australia, [2]Adelaide Medical School and [3]School of Biological Sciences University of Adelaide, Adelaide, South Australia

*Corresponding author: Maria Fuller, Genetics and Molecular Pathology, SA Pathology at Women's and Children's Hospital, 72 King William Road, North Adelaide, South Australia, 5006, Australia

Email: maria.fuller@adelaide.edu.au

Tel: +61 (0)8 8161 6741

Word Counts: Abstract 237 words, body text 3062

Key Words: Cystic fibrosis, CFTR, prevalence, screening, CF-related disorders

ABSTRACT

Objectives To determine the diagnostic yield of cystic fibrosis (CF) in symptomatic cohorts 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 2,000 reported in the CFTR gene, we hypothesised that patients will be missed and present clinically later in life.

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

Setting A single pathology service in South Australia.

Participants A total of 1,909 CF test referrals from patients with clinical suspicion indicated by respiratory and gastrointestinal manifestations, fetal 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 was 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 fetal 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.

STRENGHTS AND LIMITATIONS OF THIS STUDY

- A retrospective study to determine the diagnostic yield of 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 CFTR variants, followed by NGS that covered 98%.
- The cohort could be clearly categorised based on symptomology 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 CFTR gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR).[1,2,3] The CFTR protein forms a channel across the cell membrane and when phosphorylated by cAMP-dependent phosphokinases 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 a multi-organ disease, effecting 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] 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.[7] The CFTR channel similarly regulates hydration of the epithelial cells lining the reproductive tract. In males, 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 (CAUVD)) resulting in obstructive azoospermia.[8] Genetic variants in the CFTR gene have also been found in other forms of male infertility including non-obstructive azoospermia and oligospermia.[9]

Since its discovery in 1989, over 2,100 variants have been identified in the CFTR gene. However, not all variants have established clinical correlation. Since April 2023, 719 variants were listed as disease causing in the CFTR2 database, whereas 49 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 1 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).[10] In severe CF disease, pancreatic insufficiency comprises 85% of all patients with CF.[11] 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 (CFTR.org). In Australia,

47% of patients diagnosed with CF are homozygous for c.1521_1523delCTT, while 43% are heterozygous (ACFD Registry Annual Report 2021). Just four alleles have >1% allele frequency in the CFTR2 database (excluding c.1521_1523delCTT), 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 3 configurations: two efficient splice sites of 7T or 9T, or one inefficient site of 5T; the presence of the 5T allele results in the absence of exon 10 in ~90% of *CFTR* mRNA expressed.[12] The adjacent TG-repeats region further influences the 5T; 12TG and 13TG repeats increases the penetrance of the 5T compared with 11TG repeats.[13]

The majority of CF cases are diagnosed neonatally in Australia with 76% made in the first three months of life (ACFD Registry Annual Report 2021). This is largely due to the established newborn screening (NBS) program, while not mandatory, has a >99% participation rate.[14] About 12% of patients are diagnosed over the age of 18 years and many adults diagnosed will have been born prior to the implementation of NBS (ACFD Registry Annual Report 2021). Additionally, clinical presentation in adults is often phenotypically different to classical CF manifesting an attenuated phenotype with VCC or rare variants not covered in the *CFTR* newborn screen. This also extends to CF-related disorders, 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 (congenital absence of the vas deferens), 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 (class I – III) or a severe and mild (class IV – VI) variant, whereas CF-RD patients usually have two mild variants or a severe and mild variant (Figure 1).[15]

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

Methods

This study compiled retrospective data on *CFTR* variant analysis from symptomatic and asymptomatic patient referrals to SA Pathology for the five-year period, January 1 2018 to

December 31 2022 and was approved by the Institution's Human Research Ethics Committee (2023/GEM00020). A diagnosis of CF was made by the identification of two CF-causing variants in the CFTR gene, as dictated by the CFTR2 database.

A total of 1,909 samples were included in this study. Symptomatic cohorts comprised clinical suspicion, fetal 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 five-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).[16] A total of 1,909 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 NGS. NGS covered >98% of the CFTR gene, including copy number variation (CNV) analysis and 194 samples were tested. A summary of the testing algorithm and sample numbers are 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 sub-group. 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: < 0.05 (*), < 0.01 (**), < 0.001 (***), < 0.0001 (****).

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). Nine 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 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 seen patients with the 5T polymorphism in combination with a CF-causing variant, resulting in a CF-RD diagnosis (Table 2). Sixteen patients (3.5%) carried one variant and 35 (7.6%) the 5T polymorphism only.

Pregnant patients with FEB detected on ultrasound as well as the patient's partner, without prior 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 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 was found in 25 patients, of which 18 carried a CF-causing variant, two carried VCC variants, and five a VUS (Table 1, 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 programs, 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 five 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 in 21% of these patients.[17] 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). Although, 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. Herein, 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 was 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 males with CBAVD, 46% with CUAVD, and up to 18% with oligospermia.[9] In 30-45% of males with CBAVD, the attributable genotype is typically CF_{severe}/CF_{mild}, CF_{mild}/CF_{mild}, $CF_{severe}/5T$ and $CF_{mild}/5T$.[19] The patients in this study presented with oligospermia, azoospermia and CBAVD; a total of 461 patients were tested and 12 males (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 males with CBAVD.[20] The large deletion variant, CFTRdele2,3, in *trans* with 5T was detected in two patients and large genomic rearrangements have been reported in *trans* with 5T in males with CBAVD.[21] The rare c.2657+2_2657+3insA 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

males.[22] 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 males with CBAVD, in *trans* with c.1521_1523delCTT,[23] as is the case herein. Sixteen patients were found to be carriers of a pathogenic *CFTR* variant supporting previous studies reporting pathogenic *CFTR* variants are higher in males with fertility issues compared to fertile males. 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.[24] 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.[24]

The FEB group, which included pregnant patients and partners (with no prior history of CF) was the largest in this study with 603 patients screened, covering 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 fetus (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 prior 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 was 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; ten had variants detected, three of which were CF-causing (c.1013C>T, c.2924_2925delGA and c.3140-26A>G), 2 VCC (c.2900T>C and c.3154T>G) and five VUS (c.92G>T, c.1079C>T, c.3389G>C, c.3979G>C and 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, 1 for c.1040G>C and 1 for c.3909C>G. The systematic review by Ioannou *et al.* found 61–100% of partners of carriers sought carrier testing for CF.[25] 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.[26] While in Africa, the allele frequency of c.1521_1523delCTT is significantly lower at 20% and 17.6% in Algeria and Tunisia, respectively.[26] The allele frequency of c.1521_1523delCTT in Asia is 12–31%.[27] 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.[28] 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 (Statistics Abo. 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%.[29] In this study, we determined the allelic frequency of 5T to be 3.2% (121 of 3,818 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 to 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] 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.[30]

In conclusion, the Australian population offers a unique landscape to monitor and collate contemporary epidemiological data that reflects 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%) newborn screening program.

Funding

This study received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

MF designed the study. JM compiled and analysed the data. JM and MF interpreted the data and wrote the manuscript.

Acknowledgements

The authors would like to thank all the staff involved in the laboratory testing of CF in Genetics and Molecular Pathology at SA Pathology for their contributions.

Data availability

The individual patient data that were analysed in this study are not able to be shared for privacy and ethical reasons. The remaining data are available within the Article.

Ethics approval

This study was approved by the Women's and Children's Health Network Human Research Ethics Committee in accordance with the National Statement on Ethical Conduct in Human Research by the National Health and Medical Research Council, Australia (identifier: 2023/GEM00020, approved 28/03/2023). This retrospective study collected genotype data from patients who had consented to *CFTR* genetic testing; patient data was de-identified to retain anonymity.

References

1. Riordan JR, Rommens JM, Kerem B-s, Alon N, Rozmahel R, Grzelczak Z, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science. 1989;245(4922):1066-73.
2. Kerem B-s, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, et al. Identification of the cystic fibrosis gene: genetic analysis. Science. 1989;245(4922):1073-80.
3. Bear CE, Li C, Kartner N, Bridges RJ, Jensen TJ, Ramjeesingh M, et al. Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). Cell. 1992;68(4):809-18.
4. Collins FS. Cystic fibrosis: Molecular Biology and Therapeutic Implications. Science. 1992;256(5058):774-9.
5. Fraser-Pitt D, O'Neil D. Cystic fibrosis–a multiorgan protein misfolding disease. Future science OA. 2015;1(2).
6. Pilewski JM, Frizzell RA. Role of CFTR in airway disease. Physiological reviews. 1999;79(1):S215-S55.
7. Dorsey J, Gonska T. Bacterial overgrowth, dysbiosis, inflammation, and dysmotility in the Cystic Fibrosis intestine. Journal of Cystic Fibrosis. 2017;16:S14-S23.
8. Chillón M, Casals T, Mercier B, Bassas L, Lissens W, Silber S, et al. Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. New England Journal of Medicine. 1995;332(22):1475-80.
9. Bieniek JM, Lapin CD, Jarvi KA. Genetics of CFTR and male infertility. Translational Andrology and Urology. 2021;10(3):1391.
10. Shteinberg M, Haq IJ, Polineni D, Davies JC. Cystic fibrosis. Lancet. 2021;397(10290):2195-211.
11. Wilschanski M, Durie PR. Patterns of GI disease in adulthood associated with mutations in the CFTR gene. Gut. 2007;56(8):1153-63.
12. Chu C-S, Trapnell BC, Curristin S, Cutting GR, Crystal RG. Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. Nature genetics. 1993;3(2):151-6.
13. Groman JD, Hefferon TW, Casals T, Bassas L, Estivill X, Des Georges M, et al. Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign. The American Journal of Human Genetics. 2004;74(1):176-9.
14. White S, Mossfield T, Fleming J, Barlow-Stewart K, Ghedia S, Dickson R, et al. Expanding the Australian Newborn Blood Spot Screening Program using genomic sequencing: do we want it and are we ready? European Journal of Human Genetics. 2023:1-9.
15. Bombieri C, Claustres M, De Boeck K, Derichs N, Dodge J, Girodon E, et al. Recommendations for the classification of diseases as CFTR-related disorders. Journal of Cystic Fibrosis. 2011;10:S86-S102.
16. Deignan JL, Astbury C, Cutting GR, Del Gaudio D, Gregg AR, Grody WW, et al. CFTR variant testing: a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genetics in Medicine. 2020;22(8):1288-95.
17. Bishop MD, Freedman SD, Zielenski J, Ahmed N, Dupuis A, Martin S, et al. The cystic fibrosis transmembrane conductance regulator gene and ion channel function in patients with idiopathic pancreatitis. Human genetics. 2005;118:372-81.
18. Bombieri C, Benetazzo M, Saccomani A, Belpinati F, Gilè LS, Luisetti M, et al. Complete mutational screening of the CFTR gene in 120 patients with pulmonary disease. Human genetics. 1998;103:718-22.

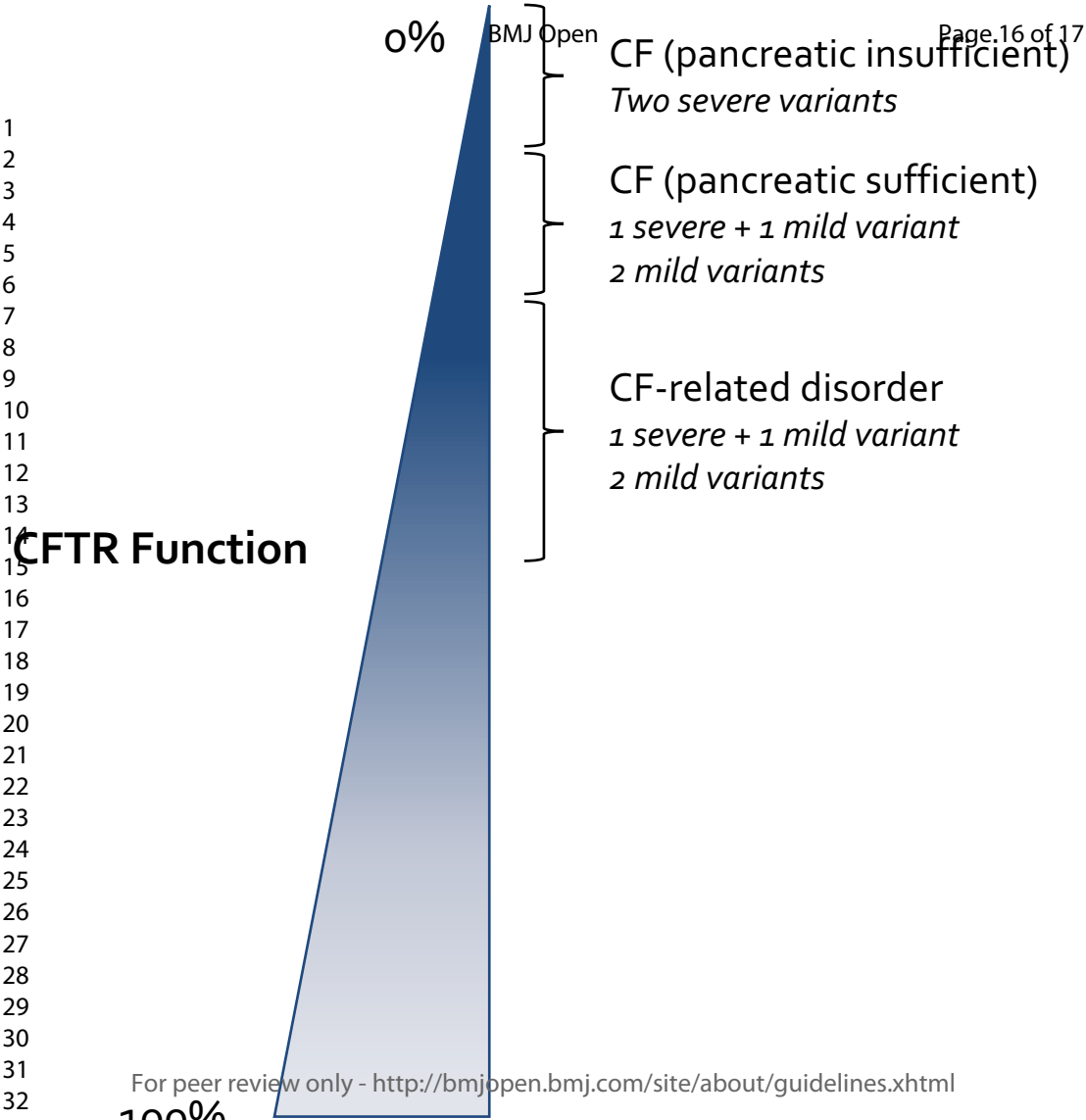
19. Giuliani R, Antonucci I, Torrente I, Grammatico P, Palka G, Stuppia L. Identification of the second CFTR mutation in patients with congenital bilateral absence of vas deferens undergoing ART protocols. Asian journal of andrology. 2010;12(6):819.
20. Dörk T, Dworniczak B, Aulehla-Scholz C, Wieczorek D, Böhm I, Mayerova A, et al. Distinct spectrum of CFTR gene mutations in congenital absence of vas deferens. Human genetics. 1997;100:365-77.
21. Ratbi I, Legendre M, Niel F, Martin J, Soufir J-C, Izard V, et al. Detection of cystic fibrosis transmembrane conductance regulator (CFTR) gene rearrangements enriches the mutation spectrum in congenital bilateral absence of the vas deferens and impacts on genetic counselling. Human reproduction. 2007;22(5):1285-91.
22. Jezequel P, Dubourg C, Le Lannou D, Odent S, Le Gall J-Y, Blayau M, et al. Molecular screening of the CFTR gene in men with anomalies of the vas deferens: identification of three novel mutations. Molecular human reproduction. 2000;6(12):1063-7.
23. De Braekeleer M, Ferec C. Mutations in the cystic fibrosis gene in men with congenital bilateral absence of the vas deferens. MHR: Basic science of reproductive medicine. 1996;2(9):669-77.
24. Yu J, Chen Z, Ni Y, Li Z. CFTR mutations in men with congenital bilateral absence of the vas deferens (CBAVD): a systemic review and meta-analysis. Human reproduction. 2012;27(1):25-35.
25. Ioannou L, McClaren BJ, Massie J, Lewis S, Metcalfe SA, Forrest L, et al. Population-based carrier screening for cystic fibrosis: a systematic review of 23 years of research. Genetics in Medicine. 2014;16(3):207-16.
26. Bobadilla JL, Macek Jr M, Fine JP, Farrell PM. Cystic fibrosis: a worldwide analysis of CFTR mutations—correlation with incidence data and application to screening. Human mutation. 2002;19(6):575-606.
27. Singh M, Rebordosa C, Bernholz J, Sharma N. Epidemiology and genetics of cystic fibrosis in a sia: in preparation for the next-generation treatments. Respirology. 2015;20(8):1172-81.
28. Zvereff VV, Faruki H, Edwards M, Friedman KJ. Cystic fibrosis carrier screening in a North American population. Genetics in Medicine. 2014;16(7):539-46.
29. Nykamp K, Truty R, Riethmaier D, Wilkinson J, Bristow SL, Aguilar S, et al. Elucidating clinical phenotypic variability associated with the polyT tract and TG repeats in CFTR. Human mutation. 2021;42(9):1165-72.
30. Deignan JL, Gregg AR, Grody WW, Guo MH, Kearney H, Monaghan KG, et al. Updated recommendations for CFTR carrier screening: A position statement of the American College of Medical Genetics and Genomics (ACMG). Genetics in Medicine. 2023:100867.

Figure Captions

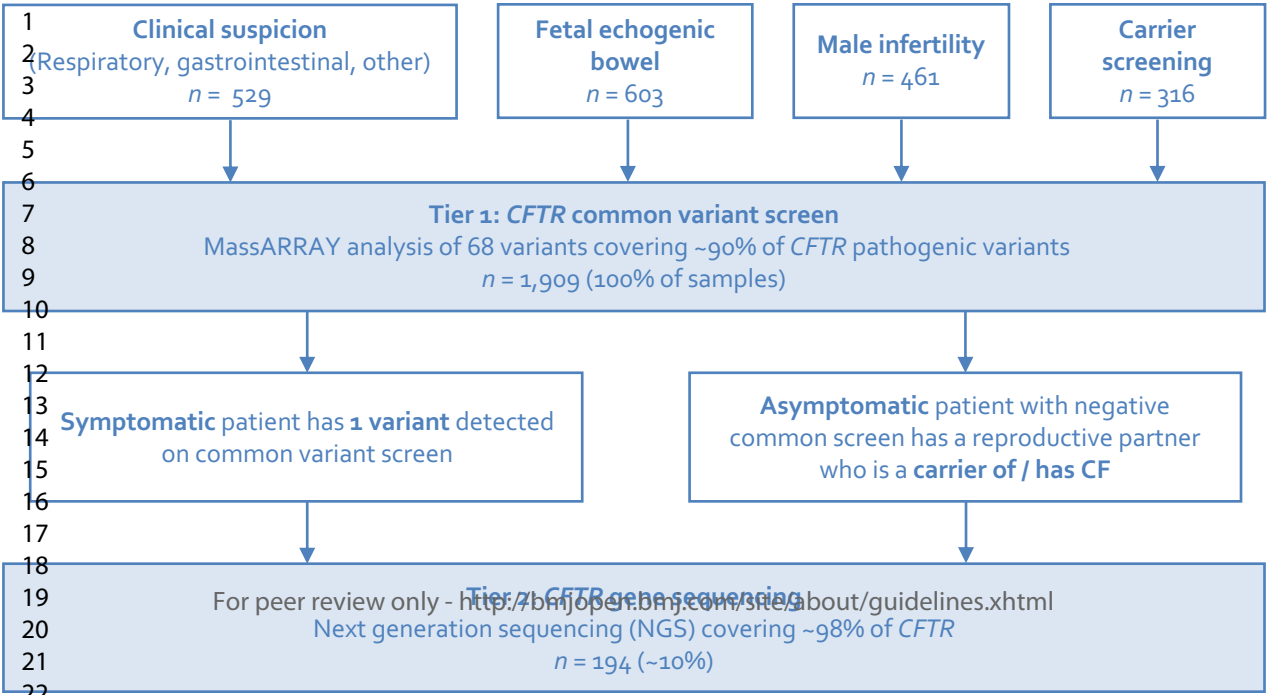
Figure 1: | Schematic diagram showing the spectrum of CF disorders corresponding to the level of CFTR function.
Variants causing a loss of, or severely reduced CFTR channel function, cause a severe form of CF (pancreatic insufficient). Variants with residual CFTR function cause a milder form of CF (pancreatic sufficient). Patients that 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.

Figure 2: Two tier screening process.
In the study period January 1 2018 to December 31 2022 1,909 samples were tested on the CFTR common variant screen from symptomatic and asymptomatic cohorts. Approximately 10% of samples were reflexed to NGS.

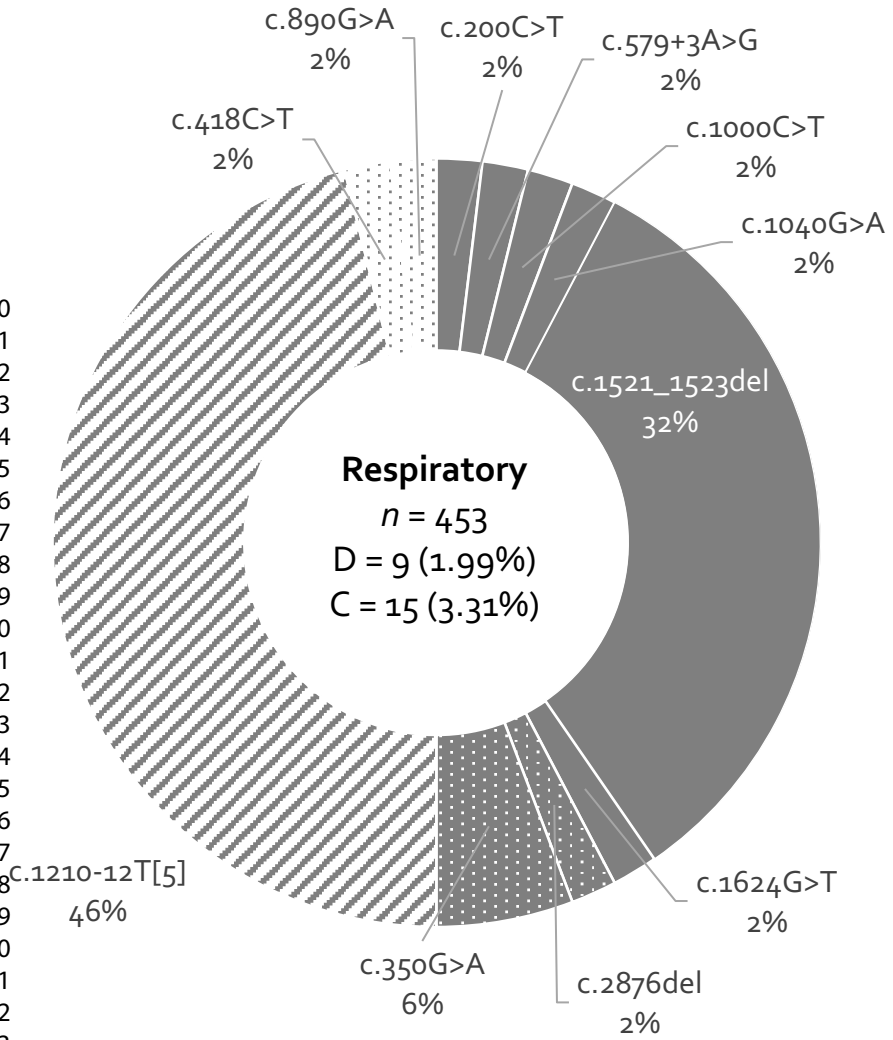
Figure 3: CFTR variants identified in study sub-groups.
Variants classified as pathogenic, likely pathogenic, varying clinical consequences (VCC) and variant of uncertain significance (VUS) that were reported for patient sub-groups.

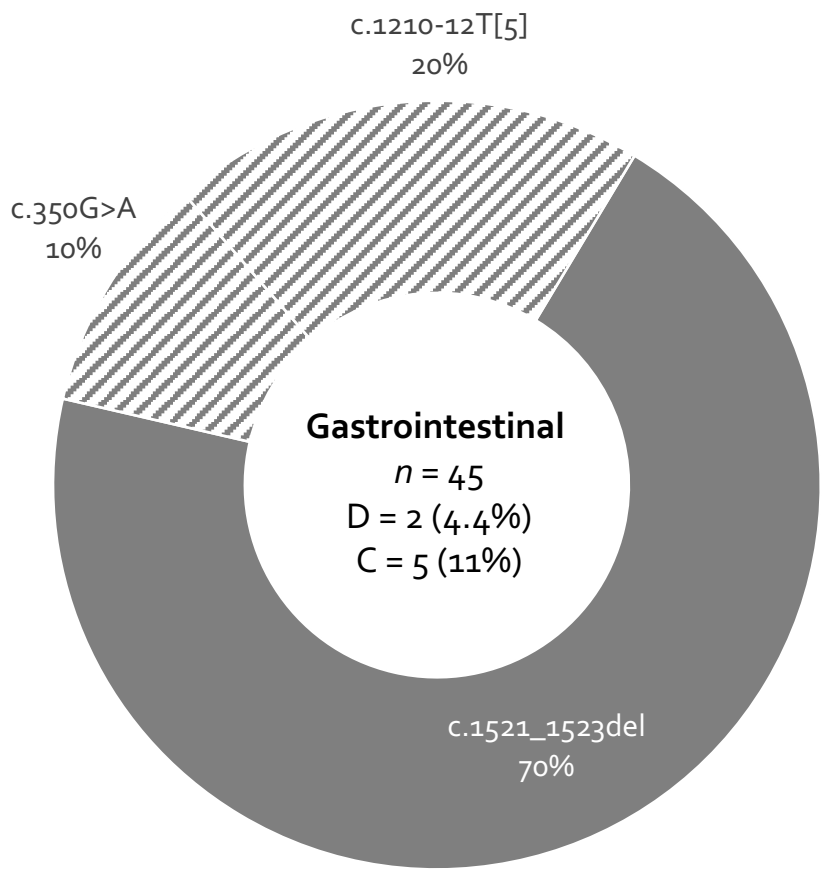


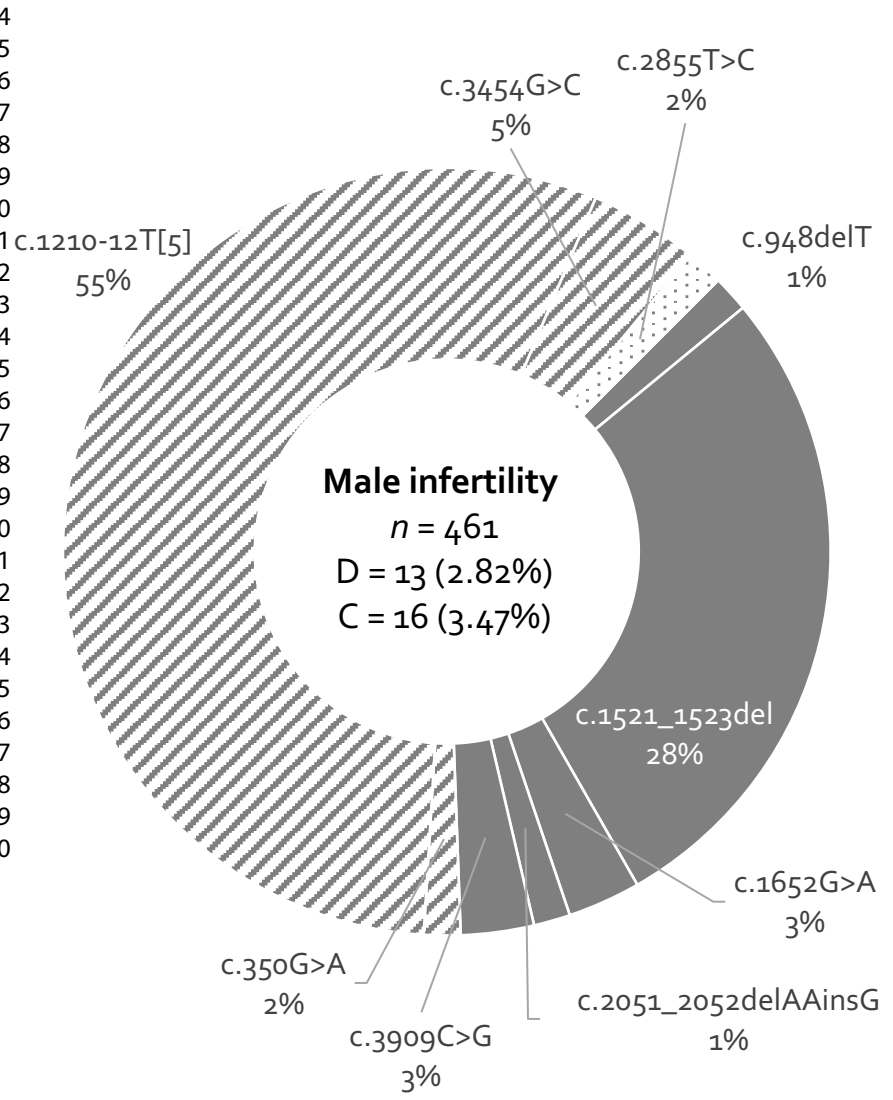
SA Pathology two-tier *CFTR* genetic analysis

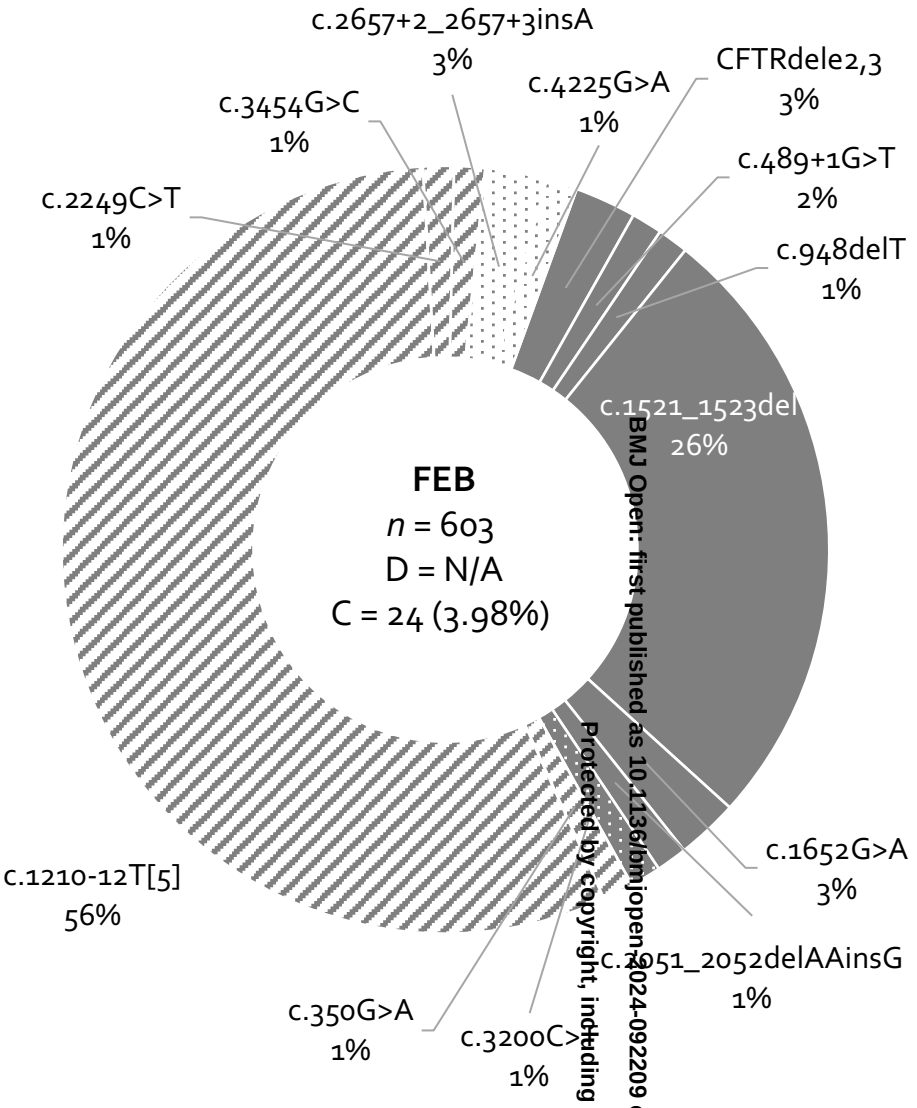


Symptomatic

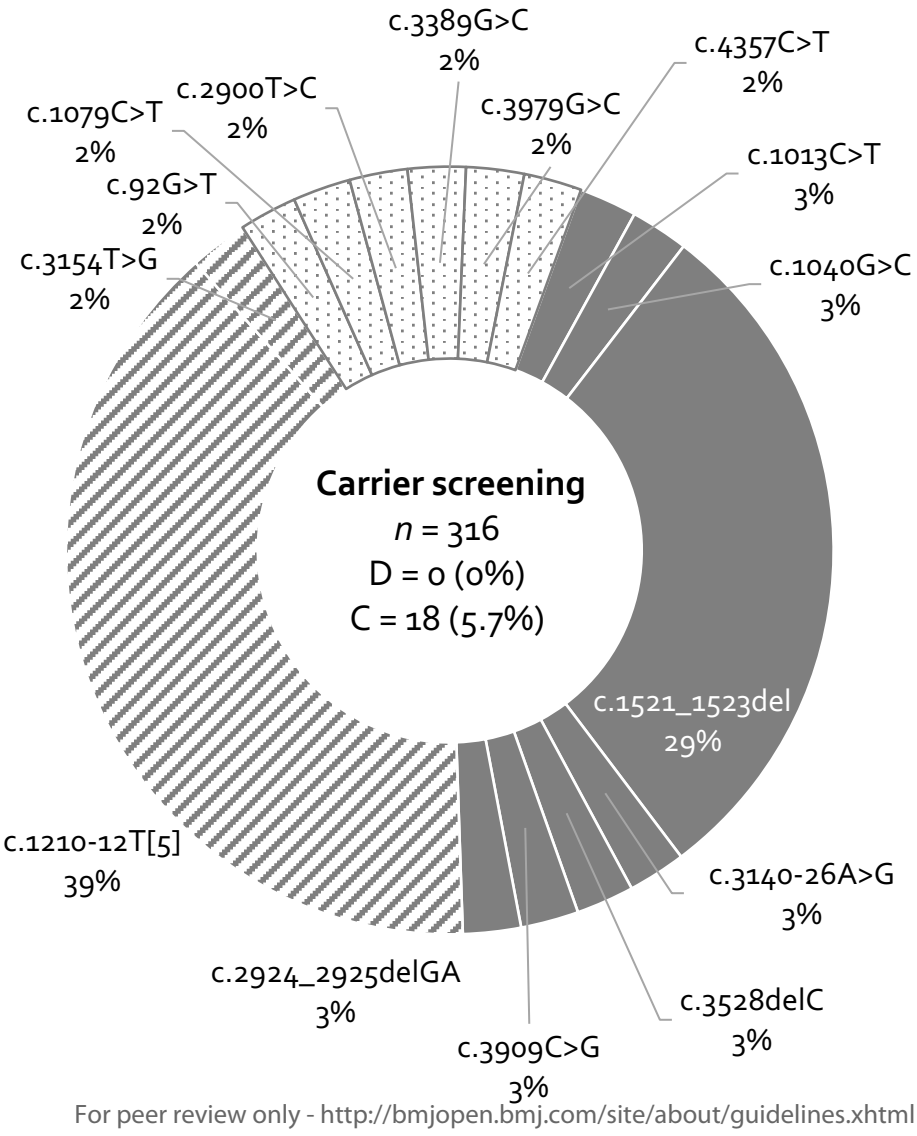








Asymptomatic



BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-092209.R1
Article Type:	Original research
Date Submitted by the Author:	02-Dec-2024
Complete List of Authors:	Markulić, Jasmina; SA Pathology, Genetics and Molecular Pathology; The University of Adelaide Faculty of Health and Medical Sciences Fuller, Maria; SA Pathology, Genetics and Molecular Pathology; The University of Adelaide Faculty of Health and Medical Sciences, ; The University of Adelaide Faculty Sciences Engineering and Technology, Biological Sciences
Primary Subject Heading:	Genetics and genomics
Secondary Subject Heading:	Epidemiology
Keywords:	Cystic fibrosis < THORACIC MEDICINE, Prevalence, GENETICS

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

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

Jasmina Markulic PhD [1,2] and Maria Fuller PhD [1,2,3*]

[1]Genetics and Molecular Pathology, SA Pathology at Women's and Children's Hospital, North Adelaide, South Australia, [2]Adelaide Medical School and [3]School of Biological Sciences University of Adelaide, Adelaide, South Australia

*Corresponding author: Maria Fuller, Genetics and Molecular Pathology, SA Pathology at Women's and Children's Hospital, 72 King William Road, North Adelaide, South Australia, 5006, Australia

Email: maria.fuller@adelaide.edu.au

Tel: +61 (0)8 8161 6741

Word Counts: Abstract 235 words, body text 3106

Key Words: Cystic fibrosis, CFTR, prevalence, screening, CF-related disorders

ABSTRACT

Objectives To determine the diagnostic yield of cystic fibrosis (CF) in 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 2,000 reported in the CFTR gene, we hypothesised that patients will be missed and present clinically later in life.

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

Setting A single pathology service in South Australia.

Participants A total of 1,909 CF test referrals from patients with clinical suspicion indicated by respiratory and gastrointestinal manifestations, fetal 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 was 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 fetal 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.

STRENGHTS AND LIMITATIONS OF THIS STUDY

- A retrospective study to determine the diagnostic yield of 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 CFTR variants, followed by NGS that covered 98%.
- The cohort could be clearly categorised based on symptomology 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 CFTR gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR).[1,2,3] The CFTR protein forms a channel across the cell membrane and when phosphorylated by cAMP-dependent phosphokinases 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 a multi-organ disease, effecting 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] 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.[7] The CFTR channel similarly regulates hydration of the epithelial cells lining the reproductive tract. In males, 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 (CAUVD)) resulting in obstructive azoospermia.[8] Genetic variants in the CFTR gene have also been found in other forms of male infertility including non-obstructive azoospermia and oligospermia.[9]

Since its discovery in 1989, over 2,100 variants have been identified in the CFTR gene. However, not all variants have established clinical correlation. Since April 2023, 719 variants were listed as disease causing in the CFTR2 database, whereas 49 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 1 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).[10] In severe CF disease, pancreatic insufficiency comprises 85% of all patients with CF.[11] 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 (CFTR.org). In Australia, 47% of patients diagnosed with CF are homozygous for c.1521_1523delCTT, while 43% are heterozygous (ACFD Registry Annual Report 2021). Just four alleles have >1% allele frequency in the CFTR2 database (excluding c.1521_1523delCTT), 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 3 configurations: two efficient splice sites of 7T or 9T, or one inefficient site of 5T; the presence of the 5T allele results in the absence of exon 10 in ~90% of *CFTR* mRNA expressed.[12] The adjacent TG-repeats region further influences the 5T; 12TG and 13TG repeats increases the penetrance of the 5T compared with 11TG repeats.[13] The 5T is therefore classified as a VCC variant by CFTR2 due to heterogenous clinical outcomes ranging from CF to CF-related diagnoses, when in combination with a CF-causing variant.

The majority of CF cases are diagnosed neonatally in Australia with 76% made in the first three months of life (ACFD Registry Annual Report 2021). This is largely due to the established newborn screening (NBS) program, while not mandatory, has a >99% participation rate.[14] About 12% of patients are diagnosed over the age of 18 years and many adults diagnosed will have been born prior to the implementation of NBS (ACFD Registry Annual Report 2021). Additionally, clinical presentation in adults is often phenotypically different to classical CF manifesting an attenuated phenotype with VCC or rare variants not covered in the *CFTR* newborn screen. This also extends to CF-related disorders, 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 (congenital absence of the vas deferens), 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 (class I – III) or a severe and mild (class IV – VI) variant, whereas CF-RD patients usually have two mild variants or a severe and mild variant (Figure 1).[15]

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

Methods

This study compiled retrospective data on *CFTR* variant analysis from symptomatic and asymptomatic patient referrals to SA Pathology for the five-year period, January 1 2018 to December 31 2022 and was approved by the Institution's Human Research Ethics Committee (2023/GEM00020). A diagnosis of CF was made by the identification of two CF-causing variants in the CFTR gene, as dictated by the CFTR2 database.

A total of 1,909 samples were included in this study. Symptomatic cohorts comprised clinical suspicion, fetal 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 five-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).[16] A total of 1,909 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 NGS. NGS covered all exons of the CFTR gene and copy number variation (CNV) analysis. 194 samples were tested by NGS. A summary of the testing algorithm and sample numbers are 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 sub-group. 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: < 0.05 (*), < 0.01 (**), < 0.001 (***), < 0.0001 (****).

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). Nine 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 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 seen patients with the 5T polymorphism in combination with a CF-causing variant, resulting in a CF-RD diagnosis (Table 2). Sixteen patients (3.5%) carried one variant and 35 (7.6%) the 5T polymorphism only.

Pregnant patients with FEB detected on ultrasound as well as the patient's partner, without prior 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 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 was found in 25 patients, of which 18 carried a CF-causing variant, two carried VCC variants, and five a VUS (Table 1, 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 programs, 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 five 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 in 21% of these patients.[17] 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). Although, 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. Herein, 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 was 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 males with CBAVD, 46% with CUAVD, and up to 18% with oligospermia.[9] In 30-45% of males with CBAVD, the attributable genotype is typically CF_{severe}/CF_{mild}, CF_{mild}/CF_{mild}, $CF_{severe}/5T$ and $CF_{mild}/5T$.[19] The patients in this study presented with oligospermia, azoospermia and CBAVD; a total of 461 patients were tested and 12 males (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 males with CBAVD.[20] The large deletion variant, CFTRdele2,3, in *trans* with 5T was detected in two patients and large genomic rearrangements have been reported in *trans* with 5T in males with CBAVD.[21] The rare c.2657+2_2657+3insA 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 males.[22] 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 males with CBAVD, in

trans with c.1521_1523delCTT,[23] as is the case herein. Sixteen patients were found to be carriers of a pathogenic *CFTR* variant supporting previous studies reporting pathogenic *CFTR* variants are higher in males with fertility issues compared to fertile males. 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.[24] 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.[24]

The FEB group, which included pregnant patients and partners (with no prior history of CF) was the largest in this study with 603 patients screened, covering 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 fetus (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 prior 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 was 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; ten had variants detected, three of which were CF-causing (c.1013C>T, c.2924_2925delGA and c.3140-26A>G), 2 VCC (c.2900T>C and c.3154T>G) and five VUS (c.92G>T, c.1079C>T, c.3389G>C, c.3979G>C and 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, 1 for c.1040G>C and 1 for c.3909C>G. The systematic review by Ioannou *et al.* found 61–100% of partners of carriers sought carrier testing for CF.[25] 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.[26] While in Africa, the allele frequency of c.1521_1523delCTT is significantly lower at 20% and 17.6% in Algeria and Tunisia, respectively.[26] The allele frequency of c.1521_1523delCTT in Asia is 12–31%.[27] 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.[28] 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 (Statistics Abo. 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%.[29] In this study, we determined the allelic frequency of 5T to be 3.2% (121 of 3,818 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 to 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] 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.[30]

In conclusion, the Australian population offers a unique landscape to monitor and collate contemporary epidemiological data that reflects 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%) newborn screening program. 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.

Funding

This study received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflict of interest

The authors declare no conflicts of interest.

Patients and Public Involvement

Patients and the public are not involved in the design, or conduct , or reporting, or dissemination plans of our research.

Author contributions

MF designed the study. JM compiled and analysed the data. JM and MF interpreted the data and wrote the manuscript. MF is the guarantor.

Acknowledgements

The authors would like to thank all the staff involved in the laboratory testing of CF in Genetics and Molecular Pathology at SA Pathology for their contributions.

Data availability

The individual patient data that were analysed in this study are not able to be shared for privacy and ethical reasons. The remaining data are available within the Article.

Ethics approval

This study was approved by the Women's and Children's Health Network Human Research Ethics Committee in accordance with the National Statement on Ethical Conduct in Human Research by the National Health and Medical Research Council, Australia (identifier: 2023/GEM00020, approved 28/03/2023). This retrospective study collected genotype data from patients who had consented to *CFTR* genetic testing; patient data was de-identified to retain anonymity.

References

1. Riordan JR, Rommens JM, Kerem B-s, Alon N, Rozmahel R, Grzelczak Z, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science. 1989;245(4922):1066-73.
2. Kerem B-s, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, et al. Identification of the cystic fibrosis gene: genetic analysis. Science. 1989;245(4922):1073-80.
3. Bear CE, Li C, Kartner N, Bridges RJ, Jensen TJ, Ramjeesingh M, et al. Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). Cell. 1992;68(4):809-18.
4. Collins FS. Cystic fibrosis: Molecular Biology and Therapeutic Implications. Science. 1992;256(5058):774-9.
5. Fraser-Pitt D, O'Neil D. Cystic fibrosis–a multiorgan protein misfolding disease. Future science OA. 2015;1(2).
6. Pilewski JM, Frizzell RA. Role of CFTR in airway disease. Physiological reviews. 1999;79(1):S215-S55.
7. Dorsey J, Gonska T. Bacterial overgrowth, dysbiosis, inflammation, and dysmotility in the Cystic Fibrosis intestine. Journal of Cystic Fibrosis. 2017;16:S14-S23.
8. Chillón M, Casals T, Mercier B, Bassas L, Lissens W, Silber S, et al. Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. New England Journal of Medicine. 1995;332(22):1475-80.
9. Bieniek JM, Lapin CD, Jarvi KA. Genetics of CFTR and male infertility. Translational Andrology and Urology. 2021;10(3):1391.
10. Shteinberg M, Haq IJ, Polineni D, Davies JC. Cystic fibrosis. Lancet. 2021;397(10290):2195-211.
11. Wilschanski M, Durie PR. Patterns of GI disease in adulthood associated with mutations in the CFTR gene. Gut. 2007;56(8):1153-63.
12. Chu C-S, Trapnell BC, Curristin S, Cutting GR, Crystal RG. Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. Nature genetics. 1993;3(2):151-6.
13. Groman JD, Hefferon TW, Casals T, Bassas L, Estivill X, Des Georges M, et al. Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign. The American Journal of Human Genetics. 2004;74(1):176-9.
14. White S, Mossfield T, Fleming J, Barlow-Stewart K, Ghedia S, Dickson R, et al. Expanding the Australian Newborn Blood Spot Screening Program using genomic sequencing: do we want it and are we ready? European Journal of Human Genetics. 2023:1-9.
15. Bombieri C, Claustres M, De Boeck K, Derichs N, Dodge J, Girodon E, et al. Recommendations for the classification of diseases as CFTR-related disorders. Journal of Cystic Fibrosis. 2011;10:S86-S102.
16. Deignan JL, Astbury C, Cutting GR, Del Gaudio D, Gregg AR, Grody WW, et al. CFTR variant testing: a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genetics in Medicine. 2020;22(8):1288-95.

17. Bishop MD, Freedman SD, Zielenski J, Ahmed N, Dupuis A, Martin S, et al. The cystic fibrosis transmembrane conductance regulator gene and ion channel function in patients with idiopathic pancreatitis. Human genetics. 2005;118:372-81.
18. Bombieri C, Benetazzo M, Saccomani A, Belpinati F, Gilè LS, Luisetti M, et al. Complete mutational screening of the CFTR gene in 120 patients with pulmonary disease. Human genetics. 1998;103:718-22.
19. Giuliani R, Antonucci I, Torrente I, Grammatico P, Palka G, Stuppia L. Identification of the second CFTR mutation in patients with congenital bilateral absence of vas deferens undergoing ART protocols. Asian journal of andrology. 2010;12(6):819.
20. Dörk T, Dworniczak B, Aulehla-Scholz C, Wieczorek D, Böhm I, Mayerova A, et al. Distinct spectrum of CFTR gene mutations in congenital absence of vas deferens. Human genetics. 1997;100:365-77.
21. Ratbi I, Legendre M, Niel F, Martin J, Soufir J-C, Izard V, et al. Detection of cystic fibrosis transmembrane conductance regulator (CFTR) gene rearrangements enriches the mutation spectrum in congenital bilateral absence of the vas deferens and impacts on genetic counselling. Human reproduction. 2007;22(5):1285-91.
22. Jezequel P, Dubourg C, Le Lannou D, Odent S, Le Gall J-Y, Blayau M, et al. Molecular screening of the CFTR gene in men with anomalies of the vas deferens: identification of three novel mutations. Molecular human reproduction. 2000;6(12):1063-7.
23. De Braekeleer M, Ferec C. Mutations in the cystic fibrosis gene in men with congenital bilateral absence of the vas deferens. MHR: Basic science of reproductive medicine. 1996;2(9):669-77.
24. Yu J, Chen Z, Ni Y, Li Z. CFTR mutations in men with congenital bilateral absence of the vas deferens (CBAVD): a systemic review and meta-analysis. Human reproduction. 2012;27(1):25-35.
25. Ioannou L, McClaren BJ, Massie J, Lewis S, Metcalfe SA, Forrest L, et al. Population-based carrier screening for cystic fibrosis: a systematic review of 23 years of research. Genetics in Medicine. 2014;16(3):207-16.
26. Bobadilla JL, Macek Jr M, Fine JP, Farrell PM. Cystic fibrosis: a worldwide analysis of CFTR mutations—correlation with incidence data and application to screening. Human mutation. 2002;19(6):575-606.
27. Singh M, Rebordosa C, Bernholz J, Sharma N. Epidemiology and genetics of cystic fibrosis in a sia: in preparation for the next-generation treatments. Respirology. 2015;20(8):1172-81.
28. Zvereff VV, Faruki H, Edwards M, Friedman KJ. Cystic fibrosis carrier screening in a North American population. Genetics in Medicine. 2014;16(7):539-46.
29. Nykamp K, Truty R, Riethmaier D, Wilkinson J, Bristow SL, Aguilar S, et al. Elucidating clinical phenotypic variability associated with the polyT tract and TG repeats in CFTR. Human mutation. 2021;42(9):1165-72.
30. Deignan JL, Gregg AR, Grody WW, Guo MH, Kearney H, Monaghan KG, et al. Updated recommendations for CFTR carrier screening: A position statement of the American College of Medical Genetics and Genomics (ACMG). Genetics in Medicine. 2023:100867.

Tables

Table 1 | Summary of CF and CF-RD diagnoses and carriers in study sub-groups.

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)
Fetal echogenic bowel	603	29 (4.81%)	24 (3.98%)	N/A*	24 (3.98%) (>0.99)
TOTAL	**1,909**	**115 (6.02%)**	**101 (5.3%)**	**25 (2.5%)**	**79 (4.1%)**

Table 1 footnote: Values in black brackets show diagnostic and carrier frequency calculated as a percentage of patients screened for that cohort. Values in grey 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 ($p < 0.05$ (*), < 0.01 (**), < 0.001 (***), < 0.0001 (****)). Exact p-values are shown in brackets in grey.
Total CF carriers calculated with patients carrying CF-causing variants only.
*3 couples were both found to be carriers; prenatal testing found all 3 fetuses diagnosed with CF.

Table 2 | Reported CF and CF-RD variant combinations in study sub-groups.

Presentation	Variant combination	Number of alleles
Clinical suspicion – respiratory	c.1521_1523del (p.Phe508del) / c.1521_1523del (p.Phe508del)	3
	c.350G>A (p.Arg117His);T5 / c.1521_1523del (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_1523del (p.Phe508del) / c.579+3A>G	1
	c.1521_1523delCTT (p.Phe508del) / c.1040G>A (p.Arg347His)	1
	c.1521_1523del (p.Phe508del) / c.2876del (p.Ala959Aspfs*9)	1
Clinical suspicion – gastrointestinal	c.350G>A (p.Arg117His);T7 / c.1521_1523del (p.Phe508del);T9	1
	c.1521_1523del (p.Phe508del) / c.1521_1523del (p.Phe508del)	1
Clinical suspicion – other	c.1521_1523del (p.Phe508del) / c.1521_1523del (p.Phe508del)	1
Male infertility	c.1521_1523del (p.Phe508del);T9 / T5	4
	c.54-5940_273+10250del21kb (CFTRdele2,3), T5/T7 *	2
	c.350G>A (p.Arg117His), T5/T7*	1
	c.489+1G>T/c.2657+2_2657+3insA (p.?), T7/T9 *	1
	c.1521_1523del (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_1523del (p.Phe508del);T9 / c.4225G>A (p.Glu1409Lys);T9	1
	c.1652G>A (p.Gly551Asp);T7 / c.2657+2_2657+3insA (p.?);T7	1
	c.2051_2052delAAinsG, T5/T7 *	1

Table 2 footnote: Red highlight denotes a CF or CF-RD diagnosis, orange highlight denotes VCC diagnosis.
*Phasing not determined.

Table 3 | 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 3,818 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
c.4357C>T	p.Arg1453Trp	R1453W		1	0.405	0.0262
			Total	**247**		

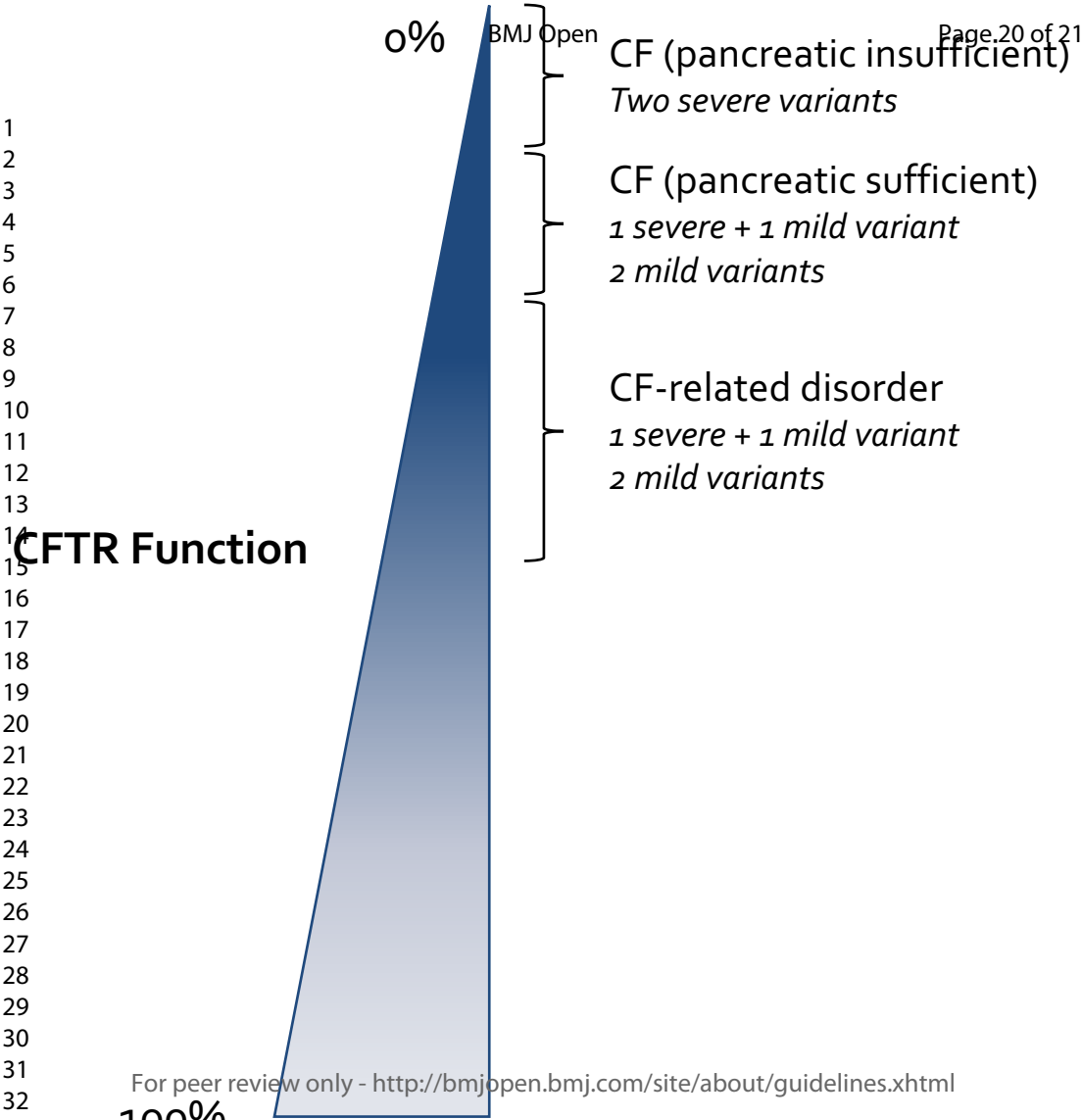
Table 3 footnote: Allele frequency and classification of CFTR variants detected in this study. Variants not included in the CFTR2 database have classification omitted. *The c.2924_2925delGA variant was added to the database in 2023 and has now been classified as CF-causing; at the time of reporting, this variant was classified as a VUS.

Figure Captions

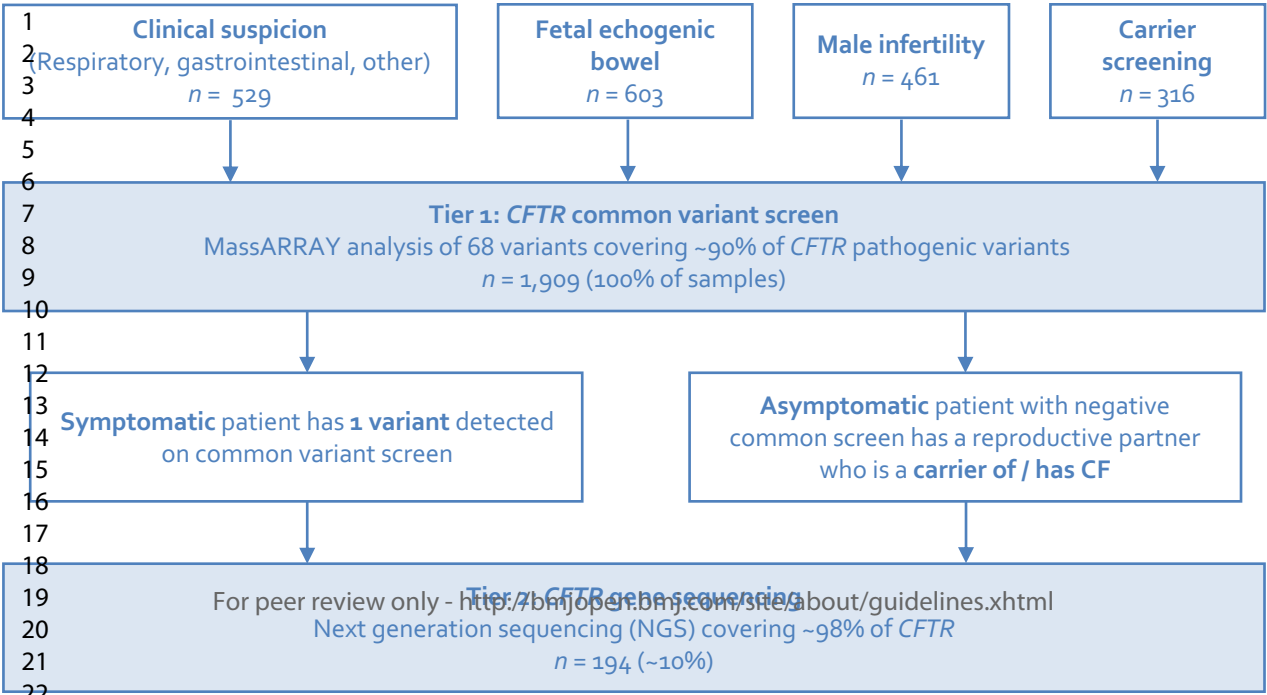
Figure 1: | Schematic diagram showing the spectrum of CF disorders corresponding to the level of CFTR function.
Variants causing a loss of, or severely reduced CFTR channel function, cause a severe form of CF (pancreatic insufficient). Variants with residual CFTR function cause a milder form of CF (pancreatic sufficient). Patients that 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.

Figure 2: Two tier screening process.
In the study period January 1 2018 to December 31 2022 1,909 samples were tested on the CFTR common variant screen from symptomatic and asymptomatic cohorts. Approximately 10% of samples were reflexed to NGS.

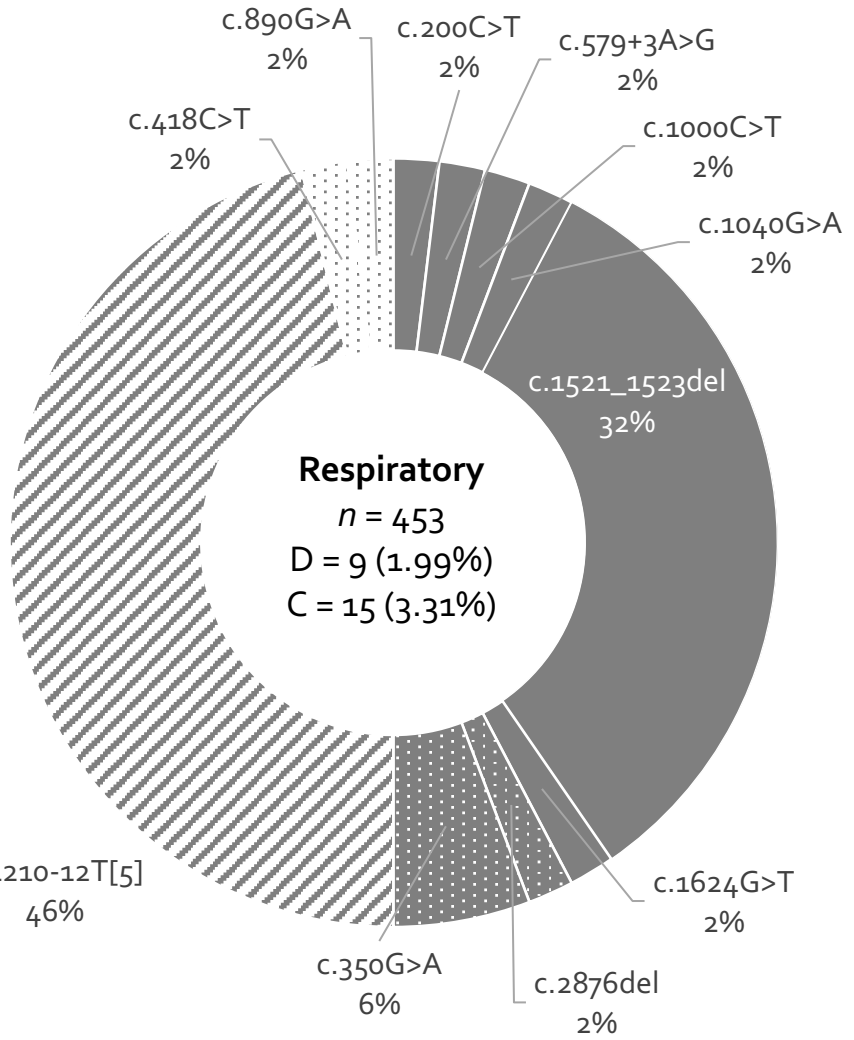
Figure 3: CFTR variants identified in study sub-groups.
Variants classified as pathogenic, likely pathogenic, varying clinical consequences (VCC) and variant of uncertain significance (VUS) that were reported for patient sub-groups.

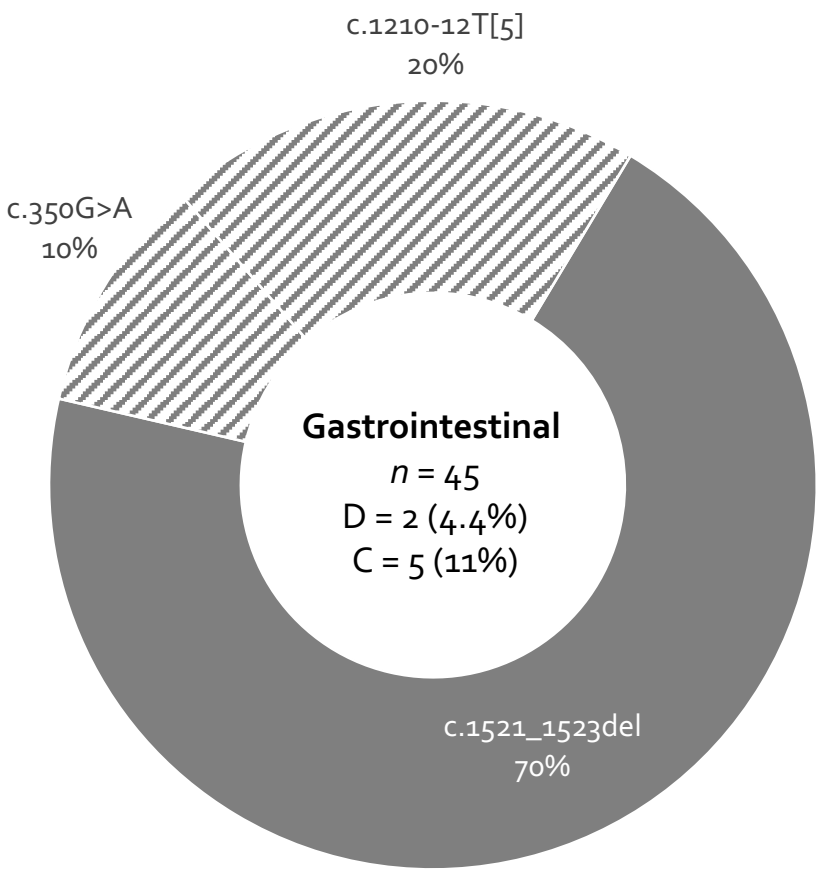


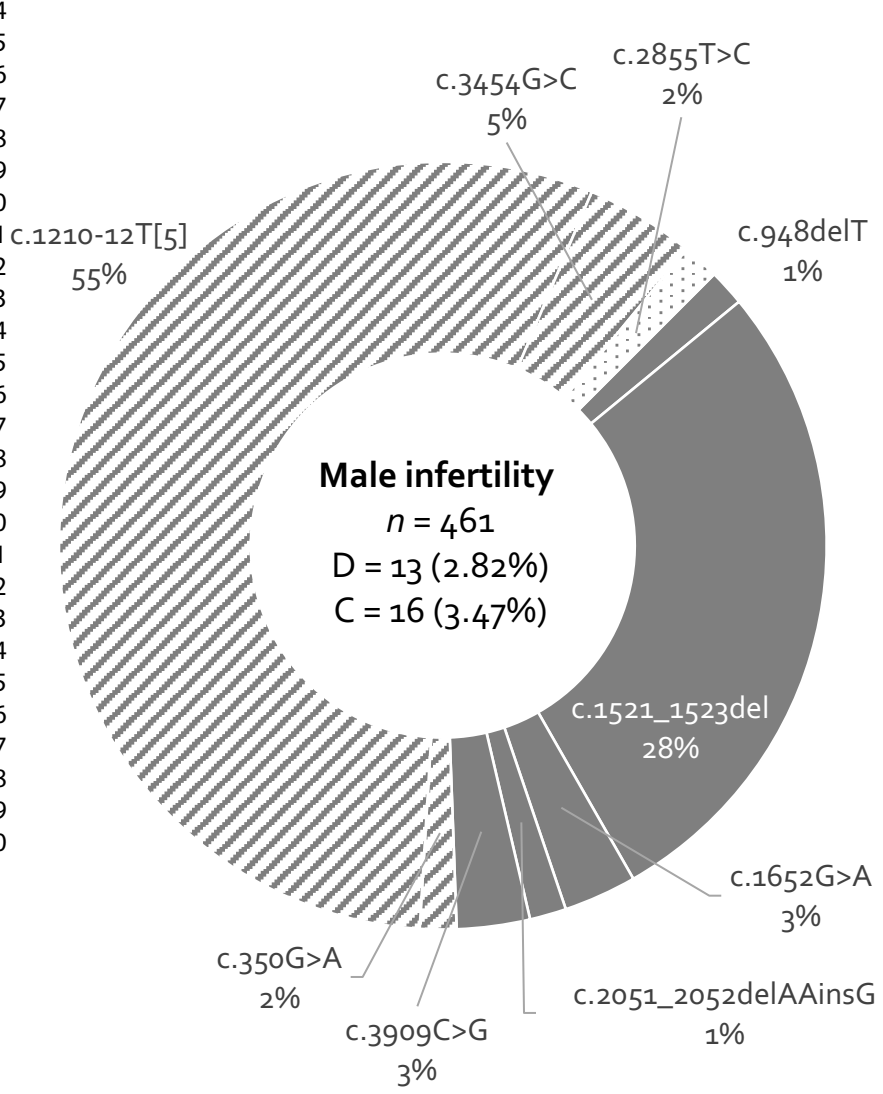
SA Pathology two-tier *CFTR* genetic analysis

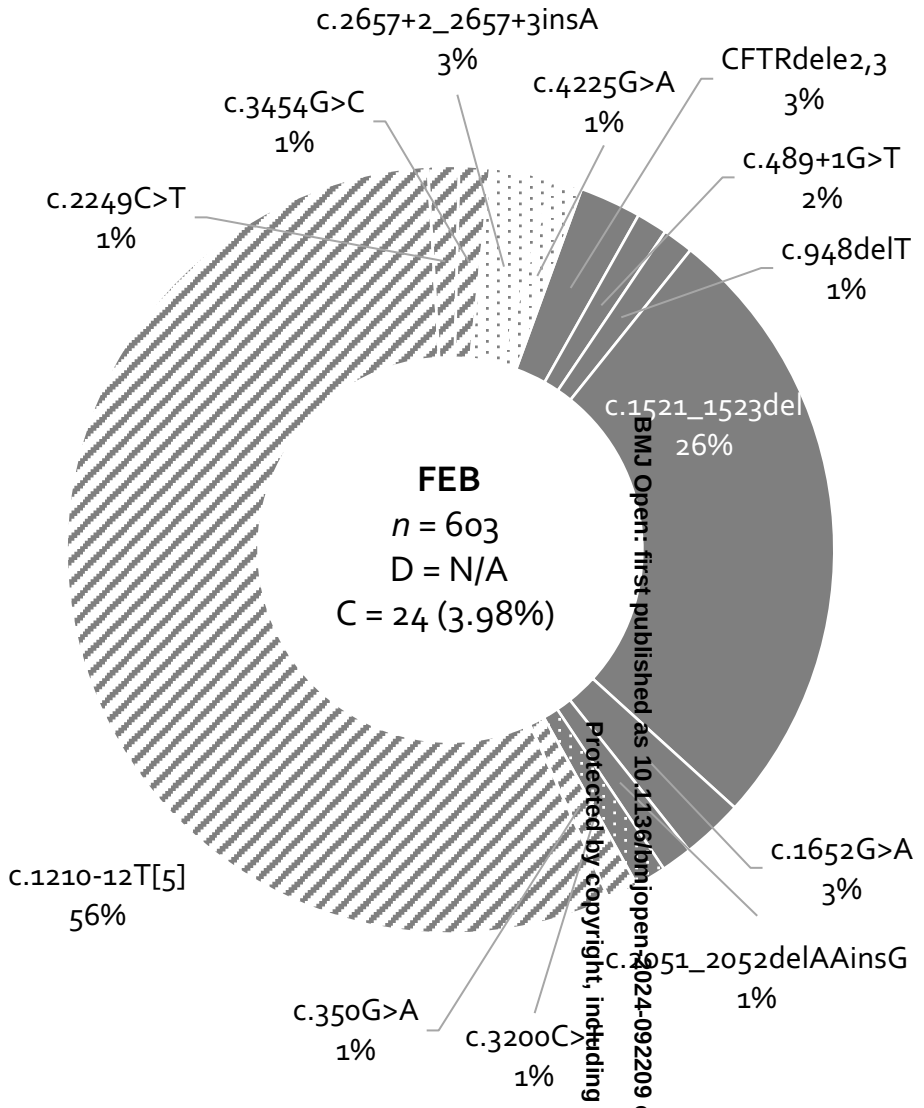


Symptomatic

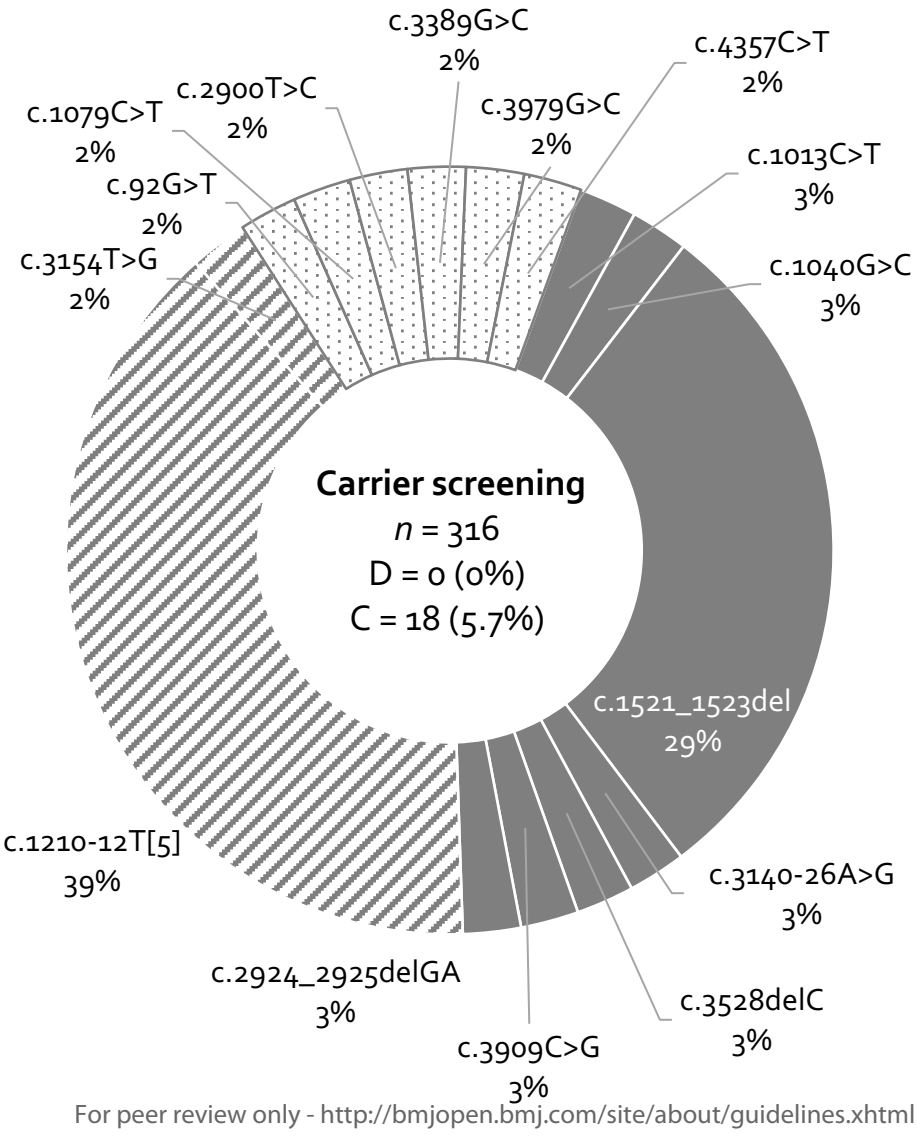








Asymptomatic



BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-092209.R2
Article Type:	Original research
Date Submitted by the Author:	14-Dec-2024
Complete List of Authors:	Markulić, Jasmina; SA Pathology, Genetics and Molecular Pathology; The University of Adelaide Faculty of Health and Medical Sciences Fuller, Maria; SA Pathology, Genetics and Molecular Pathology; The University of Adelaide Faculty of Health and Medical Sciences, ; The University of Adelaide Faculty Sciences Engineering and Technology, Biological Sciences
Primary Subject Heading:	Genetics and genomics
Secondary Subject Heading:	Epidemiology
Keywords:	Cystic fibrosis < THORACIC MEDICINE, Prevalence, GENETICS

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

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

Jasmina Markulic PhD [1,2] and Maria Fuller PhD [1,2,3*]

[1]Genetics and Molecular Pathology, SA Pathology at Women's and Children's Hospital, North Adelaide, South Australia, [2]Adelaide Medical School and [3]School of Biological Sciences University of Adelaide, Adelaide, South Australia

*Corresponding author: Maria Fuller, Genetics and Molecular Pathology, SA Pathology at Women's and Children's Hospital, 72 King William Road, North Adelaide, South Australia, 5006, Australia

Email: maria.fuller@adelaide.edu.au

Tel: +61 (0)8 8161 6741

Word Counts: Abstract 234 words, body text 3149

Key Words: Cystic fibrosis, CFTR, prevalence, screening, CF-related disorders

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 2,000 reported in the CFTR gene, we hypothesised that patients will be missed and present clinically later in life.

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

Setting A single pathology service in South Australia.

Participants A total of 1,909 CF test referrals from patients with clinical suspicion indicated by respiratory and gastrointestinal manifestations, fetal 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 was 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 fetal 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.

STRENGHTS AND LIMITATIONS OF THIS STUDY

- A retrospective study to determine the diagnostic yield of 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 CFTR variants, followed by NGS that covered 98%.
- The cohort could be clearly categorised based on symptomology 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 CFTR gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR). [1,2,3] The CFTR protein forms a channel across the cell membrane and when phosphorylated by cAMP-dependent phosphokinases 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 multi-organ disease, effecting 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 hydration of the epithelial cells lining the reproductive tract. In males, 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 (CAUVD)) resulting in obstructive azoospermia. [10-14] Genetic variants in the CFTR gene have also been found in other forms of male infertility including non-obstructive azoospermia and oligospermia. [15]

Since its discovery in 1989, over 2,100 variants have been identified in the CFTR gene. However, not all variants have established clinical correlation. Since April 2023, 719 variants were listed as disease causing in the CFTR2 database (CFTR.org), whereas 49 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 1 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), 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 3 configurations: two efficient splice sites of 7T or 9T, or 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 increases 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 diagnoses, 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 three months of life (ACFDR, Annual Report 2021). This is largely due to the established newborn screening (NBS) program, 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 prior to the implementation of NBS (ACFDR, Annual Report 2021). Additionally, clinical presentation in adults is often phenotypically different to 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, 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 (congenital absence of the vas deferens), 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 (class I – III) or a severe and mild (class 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 post-natal patients with a clinical suspicion of CF referred to our laboratory for testing. This included patients with respiratory or gastrointestinal symptoms, male infertility and fetal echogenic bowel detected on ultrasound. The aim was to determine the diagnostic yield of CF in these cohorts to guide health care practice and genetic counselling in a nation with >99% uptake of CF newborn screening.

Methods

This study compiled retrospective data on *CFTR* variant analysis from symptomatic and asymptomatic patient referrals to SA Pathology for the five-year period, January 1 2018 to December 31 2022 and was approved by the Institution's Human Research Ethics Committee (2023/GEM00020). A diagnosis of CF was made by the identification of two CF-causing variants in the CFTR gene, as dictated by the CFTR2 database.

A total of 1,909 samples were included in this study. Symptomatic cohorts comprised clinical suspicion, fetal 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 five-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 1,909 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 NGS. NGS covered all exons of the CFTR gene and copy number variation (CNV) analysis. 194 samples were tested by NGS. A summary of the testing algorithm and sample numbers are 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 sub-group. 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: < 0.05 (*), < 0.01 (**), < 0.001 (***), < 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). Nine 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 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). Sixteen patients (3.5%) carried one variant and 35 (7.6%) the 5T polymorphism only.

Pregnant patients with FEB detected on ultrasound as well as the patient's partner, without prior 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 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 was found in 25 patients, of which 18 carried a CF-causing variant, two carried VCC variants, and five a VUS (Table 1, 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 programs, 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 five 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 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). Although, 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. Herein, 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 was 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 males with CBAVD, 46% with CUAVD, and up to 18% with oligospermia. [15] In 30-45% of males 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 males (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 males with CBAVD. [31] The large deletion variant, c.54-5940_273+10250del21kb (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 males with CBAVD. [32] The rare c.2657+2_2657+3insA 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 males. [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 males with CBAVD, in *trans* with c.1521_1523delCTT, as is the case herein. [34] Sixteen patients were found to be carriers of a pathogenic *CFTR* variant supporting previous studies reporting pathogenic *CFTR* variants are higher in males with fertility issues compared to fertile males. 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 prior history of CF) was the largest in this study with 603 patients screened, covering 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 fetus (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 prior 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 was 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; ten had variants detected, three of which were CF-causing (c.1013C>T, c.2924_2925delGA and c.3140-26A>G), 2 VCC (c.2900T>C and c.3154T>G) and five VUS (c.92G>T, c.1079C>T, c.3389G>C, c.3979G>C and 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>C and 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 3,818 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 to 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 reflects 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%) newborn screening program. 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.

Funding

This study received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

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.

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.

Data availability

The individual patient data that were analysed in this study are not able to be shared for privacy and ethical reasons. The remaining data are available within the Article.

Ethics approval

This study was approved by the Women's and Children's Health Network Human Research Ethics Committee in accordance with the National Statement on Ethical Conduct in Human Research by the National Health and Medical Research Council, Australia (identifier: 2023/GEM00020, approved 28/03/2023). This retrospective study collected genotype data from patients who had consented to *CFTR* genetic testing; patient data was de-identified to retain anonymity.

References

1 Riordan, J. R. *et al.* Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* **245**, 1066-1073 (1989).
2 Kerem, B.-s. *et al.* Identification of the cystic fibrosis gene: genetic analysis. *Science* **245**, 1073-1080 (1989).
3 Bear, C. E. *et al.* Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). *Cell* **68**, 809-818 (1992).
4 Collins, F. S. Cystic fibrosis: Molecular Biology and Therapeutic Implications. *Science* **256**, 774-779 (1992).
5 Fraser-Pitt, D. & O'Neil, D. Cystic fibrosis–a multiorgan protein misfolding disease. *Future science OA* **1** (2015).
6 Pilewski, J. M. & Frizzell, R. A. Role of CFTR in airway disease. *Physiological reviews* **79**, S215-S255 (1999).
7 Collawn, J. F. & Matalon, S. CFTR and lung homeostasis. *American Journal of Physiology-Lung Cellular and Molecular Physiology* (2014).
8 Dorsey, J. & Gonska, T. Bacterial overgrowth, dysbiosis, inflammation, and dysmotility in the Cystic Fibrosis intestine. *Journal of Cystic Fibrosis* **16**, S14-S23 (2017).
9 Park, R. W. & Grand, R. J. Gastrointestinal manifestations of cystic fibrosis: a review. *Gastroenterology* **81**, 1143-1161 (1981).
10 Chillón, M. *et al.* Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. *New England Journal of Medicine* **332**, 1475-1480 (1995).

11 Cuppens, H. & Cassiman, J. J. CFTR mutations and polymorphisms in male infertility. *International journal of andrology* **27**, 251-256 (2004).

12 Chan, H. C. *et al.* The cystic fibrosis transmembrane conductance regulator in reproductive health and disease. *The Journal of physiology* **587**, 2187-2195 (2009).

13 Chen, H., Ruan, Y. C., Xu, W. M., Chen, J. & Chan, H. C. Regulation of male fertility by CFTR and implications in male infertility. *Human reproduction update* **18**, 703-713 (2012).

14 De Souza, D. A. S., Faucz, F. R., Pereira-Ferrari, L., Sotomaior, V. S. & Raskin, S. Congenital bilateral absence of the vas deferens as an atypical form of cystic fibrosis: reproductive implications and genetic counseling. *Andrology* **6**, 127-135 (2018).

15 Bieniek, J. M., Lapin, C. D. & Jarvi, K. A. Genetics of CFTR and male infertility. *Translational Andrology and Urology* **10**, 1391 (2021).

16 Bareil, C. & Bergougnoux, A. CFTR gene variants, epidemiology and molecular pathology. *Archives de Pédiatrie* **27**, eS8-eS12 (2020).

17 Shteinberg, M., Haq, I. J., Polineni, D. & Davies, J. C. Cystic fibrosis. *Lancet* **397**, 2195-2211, doi:10.1016/s0140-6736(20)32542-3 (2021).

18 Wilschanski, M. & Durie, P. R. Patterns of GI disease in adulthood associated with mutations in the CFTR gene. *Gut* **56**, 1153-1163 (2007).

19 Chu, C.-S., Trapnell, B. C., Curristin, S., Cutting, G. R. & Crystal, R. G. Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. *Nature genetics* **3**, 151-156 (1993).

20 Niksic, M., Romano, M., Buratti, E., Pagani, F. & Baralle, F. E. Functional analysis of cis-acting elements regulating the alternative splicing of human CFTR exon 9. *Human molecular genetics* **8**, 2339-2349 (1999).

21 Groman, J. D. *et al.* Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign. *The American Journal of Human Genetics* **74**, 176-179 (2004).

22 Kerem, E. *et al.* A cystic fibrosis transmembrane conductance regulator splice variant with partial penetrance associated with variable cystic fibrosis presentations. *American journal of respiratory and critical care medicine* **155**, 1914-1920 (1997).

23 Tosco, A. *et al.* Clinical outcomes of a large cohort of individuals with the F508del/5T; TG12 CFTR genotype. *Journal of Cystic Fibrosis* **21**, 850-855 (2022).

24 White, S. *et al.* Expanding the Australian Newborn Blood Spot Screening Program using genomic sequencing: do we want it and are we ready? *European Journal of Human Genetics*, 1-9 (2023).

25 Terlizzi, V. & Farrell, P. M. Update on advances in cystic fibrosis towards a cure and implications for primary care clinicians. *Current Problems in Pediatric and Adolescent Health Care*, 101637 (2024).

26 Taccetti, G. *et al.* Clinical and genotypical features of false-negative patients in 26 years of cystic fibrosis neonatal screening in Tuscany, Italy. *Diagnostics* **10**, 446 (2020).

27 Bombieri, C. *et al.* Recommendations for the classification of diseases as CFTR-related disorders. *Journal of Cystic Fibrosis* **10**, S86-S102 (2011).

28 Deignan, J. L. *et al.* CFTR variant testing: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine* **22**, 1288-1295 (2020).

29 Bishop, M. D. *et al.* The cystic fibrosis transmembrane conductance regulator gene and ion channel function in patients with idiopathic pancreatitis. *Human genetics* **118**, 372-381 (2005).

30 Giuliani, R. *et al.* Identification of the second CFTR mutation in patients with congenital bilateral absence of vas deferens undergoing ART protocols. *Asian journal of andrology* **12**, 819 (2010).
31 Dörk, T. *et al.* Distinct spectrum of CFTR gene mutations in congenital absence of vas deferens. *Human genetics* **100**, 365-377 (1997).
32 Ratbi, I. *et al.* Detection of cystic fibrosis transmembrane conductance regulator (CFTR) gene rearrangements enriches the mutation spectrum in congenital bilateral absence of the vas deferens and impacts on genetic counselling. *Human reproduction* **22**, 1285-1291 (2007).
33 Jezequel, P. *et al.* Molecular screening of the CFTR gene in men with anomalies of the vas deferens: identification of three novel mutations. *Molecular human reproduction* **6**, 1063-1067 (2000).
34 De Braekeleer, M. & Ferec, C. Mutations in the cystic fibrosis gene in men with congenital bilateral absence of the vas deferens. *MHR: Basic science of reproductive medicine* **2**, 669-677 (1996).
35 Yu, J., Chen, Z., Ni, Y. & Li, Z. CFTR mutations in men with congenital bilateral absence of the vas deferens (CBAVD): a systemic review and meta-analysis. *Human reproduction* **27**, 25-35 (2012).
36 Ioannou, L. *et al.* Population-based carrier screening for cystic fibrosis: a systematic review of 23 years of research. *Genetics in Medicine* **16**, 207-216 (2014).
37 Bobadilla, J. L., Macek Jr, M., Fine, J. P. & Farrell, P. M. Cystic fibrosis: a worldwide analysis of CFTR mutations—correlation with incidence data and application to screening. *Human mutation* **19**, 575-606 (2002).
38 Singh, M., Rebordosa, C., Bernholz, J. & Sharma, N. Epidemiology and genetics of cystic fibrosis in a sia: in preparation for the next-generation treatments. *Respirology* **20**, 1172-1181 (2015).
39 Zvereff, V. V., Faruki, H., Edwards, M. & Friedman, K. J. Cystic fibrosis carrier screening in a North American population. *Genetics in Medicine* **16**, 539-546 (2014).
40 Nykamp, K. *et al.* Elucidating clinical phenotypic variability associated with the polyT tract and TG repeats in CFTR. *Human mutation* **42**, 1165-1172 (2021).
41 Deignan, J. L. *et al.* Updated recommendations for CFTR carrier screening: A position statement of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*, 100867 (2023).

Tables

Table 1 | Summary of CF and CF-RD diagnoses and carriers in study sub-groups.

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)
Fetal echogenic bowel	603	29 (4.81%)	24 (3.98%)	N/A*	24 (3.98%) (>0.99)
TOTAL	**1,909**	**115 (6.02%)**	**101 (5.3%)**	**25 (2.5%)**	**79 (4.1%)**

Table 1 footnote: Values in black brackets show diagnostic and carrier frequency calculated as a percentage of patients screened for that cohort. Values in grey 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 ($p < 0.05$ (*), < 0.01 (**), < 0.001 (***), < 0.0001 (****)). Exact p-values are shown in brackets in grey.
Total CF carriers calculated with patients carrying CF-causing variants only.
*3 couples were both found to be carriers; prenatal testing found all 3 fetuses diagnosed with CF.

Table 2 | Reported CF and CF-RD variant combinations in study sub-groups.

Presentation	Variant combination	Number of alleles
Clinical suspicion – respiratory	c.1521_1523del (p.Phe508del) / c.1521_1523del (p.Phe508del)	3
	c.350G>A (p.Arg117His);T5 / c.1521_1523del (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_1523del (p.Phe508del) / c.579+3A>G	1
	c.1521_1523delCTT (p.Phe508del) / c.1040G>A (p.Arg347His)	1
	c.1521_1523del (p.Phe508del) / c.2876del (p.Ala959Aspfs*9)	1
Clinical suspicion – gastrointestinal	c.350G>A (p.Arg117His);T7 / c.1521_1523del (p.Phe508del);T9	1
	c.1521_1523del (p.Phe508del) / c.1521_1523del (p.Phe508del)	1
Clinical suspicion – other	c.1521_1523del (p.Phe508del) / c.1521_1523del (p.Phe508del)	1
Male infertility	c.1521_1523del (p.Phe508del);T9 / T5	4
	c.54-5940_273+10250del21kb (CFTRdele2,3), T5/T7 *	2
	c.350G>A (p.Arg117His), T5/T7*	1
	c.489+1G>T/c.2657+2_2657+3insA (p.?), T7/T9 *	1
	c.1521_1523del (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_1523del (p.Phe508del);T9 / c.4225G>A (p.Glu1409Lys);T9	1
	c.1652G>A (p.Gly551Asp);T7 / c.2657+2_2657+3insA (p.?);T7	1
	c.2051_2052delAAinsG, T5/T7 *	1

Table 2 footnote: Red highlight denotes a CF or CF-RD diagnosis, orange highlight denotes VCC diagnosis.
*Phasing not determined.

Table 3 | 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 3,818 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
c.4357C>T	p.Arg1453Trp	R1453W		1	0.405	0.0262
			Total	**247**		

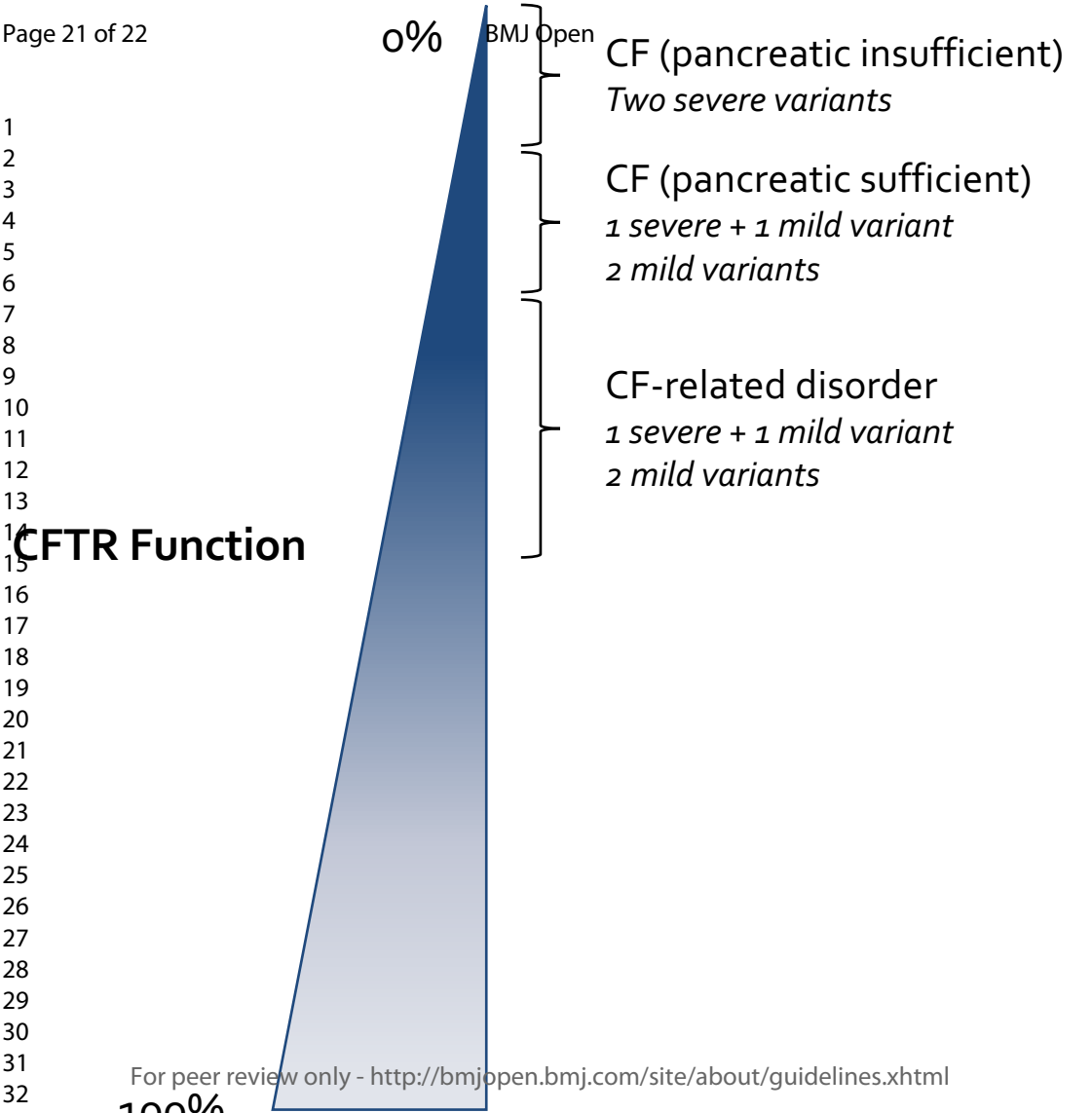
Table 3 footnote: Allele frequency and classification of CFTR variants detected in this study. Variants not included in the CFTR2 database have classification omitted. *The c.2924_2925delGA variant was added to the database in 2023 and has now been classified as CF-causing; at the time of reporting, this variant was classified as a VUS.

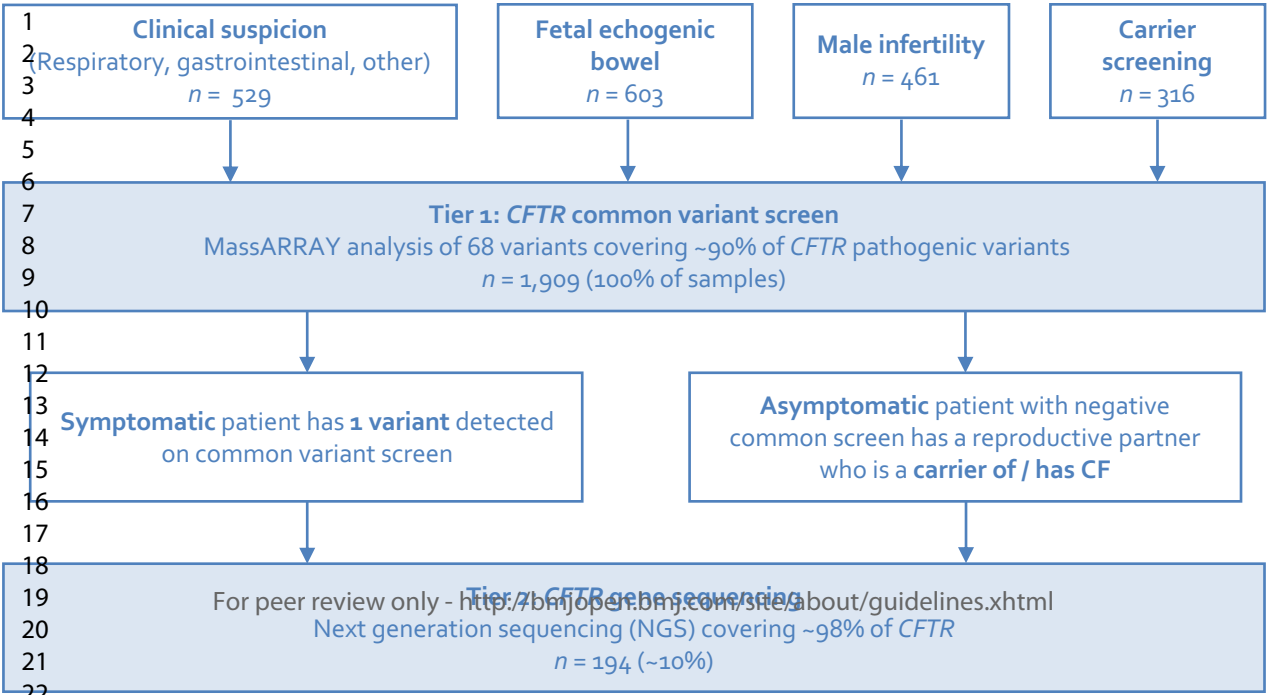
Figure Captions

Figure 1: | Schematic diagram showing the spectrum of CF disorders corresponding to the level of 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 that 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.

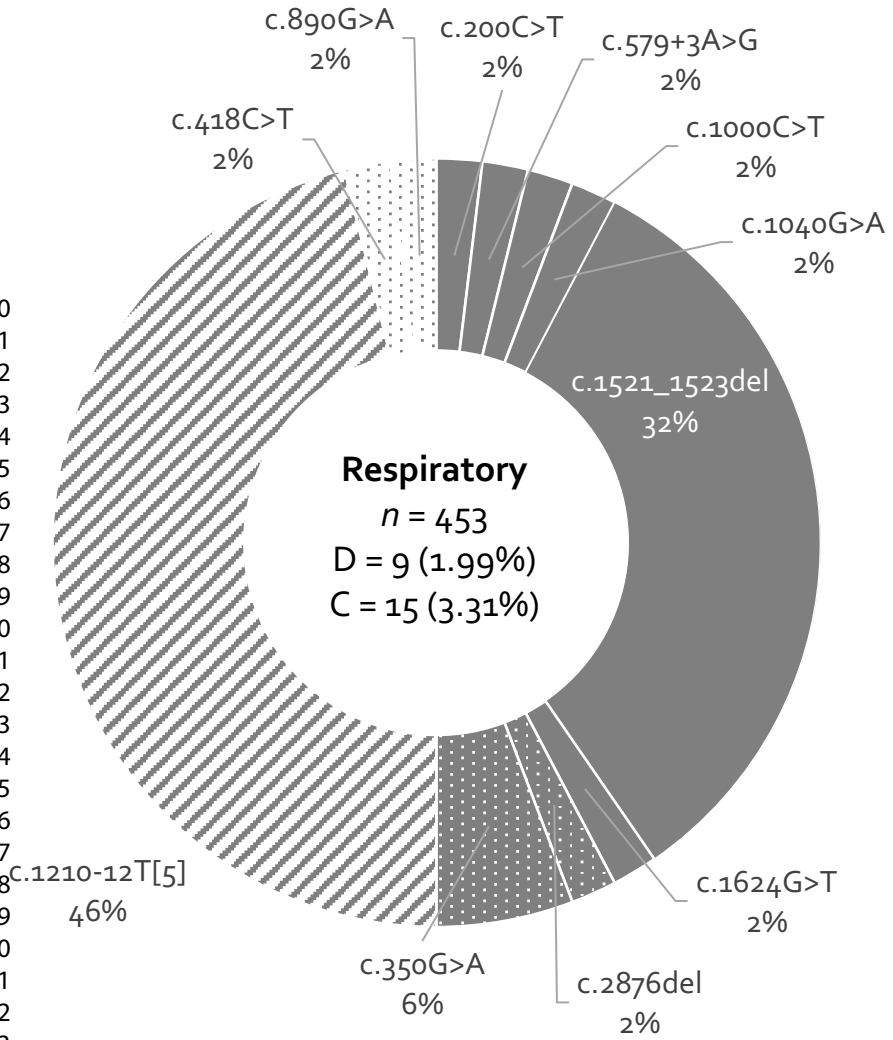
Figure 2: Two tier screening process.
In the study period January 1 2018 to December 31 2022 1,909 samples were tested on the CFTR common variant screen from symptomatic and asymptomatic cohorts. Approximately 10% of samples were reflexed to NGS.

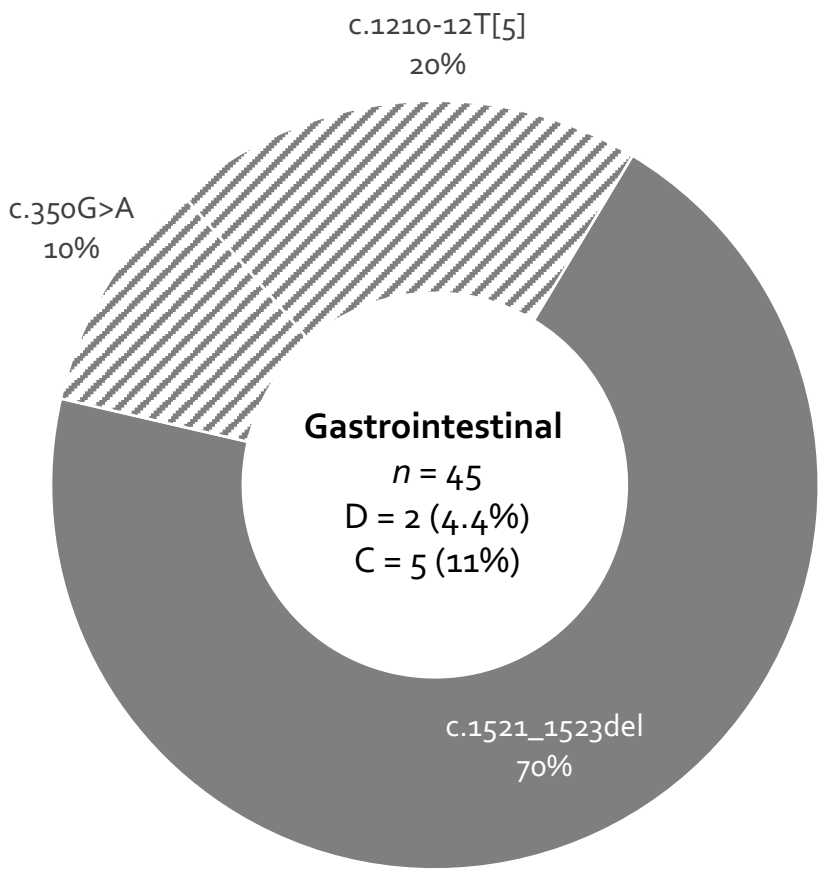
Figure 3: CFTR variants identified in study sub-groups.
Variants classified as pathogenic, likely pathogenic, varying clinical consequences (VCC) and variant of uncertain significance (VUS) that were reported for patient sub-groups.

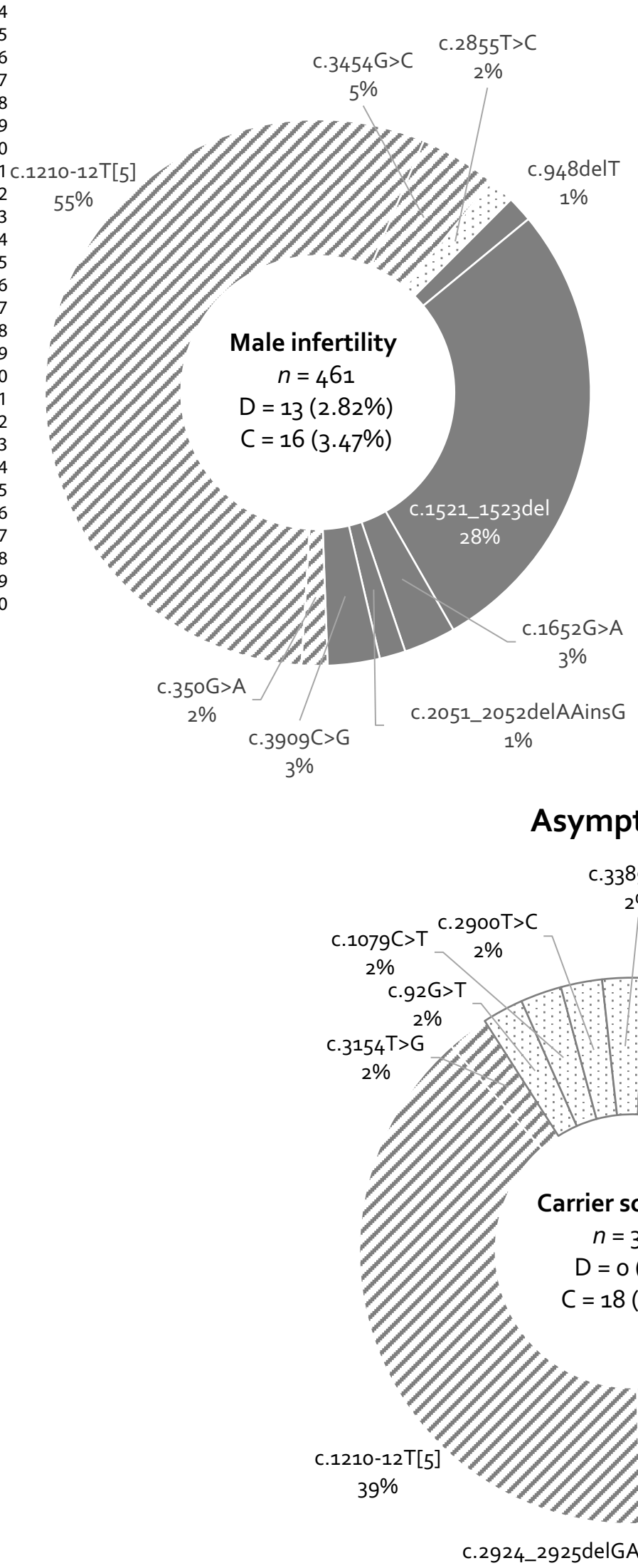


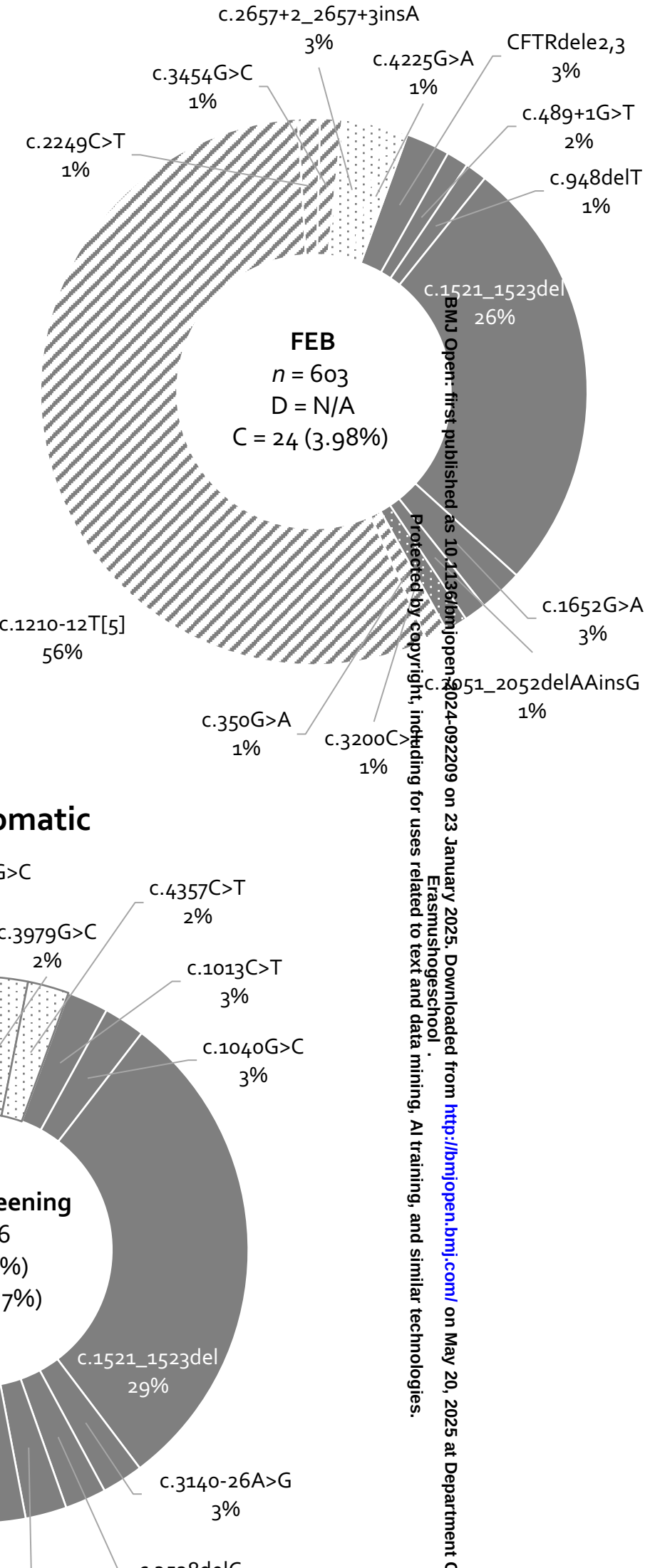


Symptomatic









Asymptomatic