CLINICAL ALERT

Cystic fibrosis in South Africa: A changing diagnostic paradigm

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Cystic fibrosis (CF), one of the most commonly observed and diagnosed fatal monogenic disorders globally, was initially thought to affect individuals of Caucasian/European descent almost exclusively. It is increasingly appreciated, however, that non-Caucasian populations are also affected by this condition. Although this has been known in South Africa (SA) for over two decades, a large disparity still exists in data pertaining to the different population groups in the country. This article seeks to highlight existing published data on CF in SA populations and reflects on the means through which these have been generated over the years. Additionally, the article briefly discusses the consequences of incomplete data and how this could potentially be addressed in the future through innovative and collaborative approaches.

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Cystic fibrosis (CF), one of the most commonly diagnosed monogenic disorders, has been well researched in many nations. Primarily affecting the mucus-secreting organs of the body, such as the lungs, pancreas, liver and intestinal tract, no cure has been developed for this fatal disorder. Nevertheless, assisted by large-scale research efforts and advances in sequencing technologies, several countries, including Australia, Belgium, Canada, France, Germany, the UK, The Netherlands and the USA, have successfully established CF registries. These registries have had a positive impact on the management and therefore on the quality of life of patients with CF and have also provided benefit in terms of reducing the costs associated with their care.[1-3]

In countries in which CF research has been well established and where CF registries and/or newborn screening programmes exist, median survival rates for patients with CF have increased steadily year on year. Currently the median age of survival is 52 years in Canada,[4] 42 years in the USA,[5] 40 years in Europe[6] and 27 years in Australia.[7] In South Africa (SA), the life expectancy of a CF patient, as reported in 2008, was less than 21 years.[8] In comparison, life expectancy for Canadian, American, French, British and Australian CF patients in the same year was approximately 30, 37, 28, 27 and 30 years, respectively.[9-13] Westwood[8] also highlighted the difference that existed between the median age of death in white individuals with CF, i.e. of Caucasian/European descent (25.8 years), and patients of mixed race (20.5 years) in the Western Cape Province of SA. Nevertheless, very little is known about causative CF variants in SA and other African nations.[14,15] This difference is due in part to the widespread and incorrect belief that CF only affects 'white' populations – a notion that is increasingly being shown to be incorrect.[16,17]

Consequences of an age-old problem and potential solutions

The initial assumption that CF could only affect white South Africans was in part based on the observed disparity in mutation detection rates between population groups. Variations in mutation detection rates have been influenced further by the fact that many CF-causing variants are population specific. Currently, more is known about CF mutations found in the well-characterised white SA population than any of the other affected population groups. As a result, causative variants are typically identified in 83% of white SA CF patients, while CF-associated variants have previously only been identified in 55% and 21% of mixed-race and black SA CF patients, respectively.[18] Screening panels initially used to diagnose CF in SA patients were based on variants that are common to European populations, and while attempts have been made to collect larger numbers of CF samples in other patient groups in SA, few publications addressing the chasm in variant data exist.

A lack of CF data in non-Caucasoid population groups is common to SA and other African countries. There are no patient registries for this disease on the African continent, which means that African CF patients cannot benefit from clinical interventions planned on the basis of trends in registry data. Despite the unparalleled genomic diversity that exists in African populations, African genomes have been vastly understudied.[19] This is illustrated in patients in whom genetic testing returns false-negative results because the CF-causing mutations have not been fully characterised in their population(s). In some cases, this problem has been resolved through the application of gene sequencing methods.[20,21] Adding further complexity to existing information is the fact that a significant proportion of reported SA CF data were published over 10 years ago (Table 1). Altogether, when considering the lack of data and the absence of an SA newborn screening programme, making a quick and accurate molecular diagnosis of CF is difficult in many cases, and assessing the health of the local CF population in order to propose and implement the interventions needed to improve prognosis is challenging.

Early detection of CF has repeatedly been shown to be of benefit to patients and public healthcare systems.[30,31] For instance, in 2012, 65%, 63%, and 50% of all new CF cases in Australia, France and The Netherlands, respectively, were detected through neonatal screening

Table 1. Methods employed to detect CF-associated variants in SA CF patients

Authors	Publication year	Reference	Method(s) used to detect CF variant(s)
Denter	1992	22	Selective screening for the ΔF508 variant only
Herbert and Retief	1992	23	Selective screening for the ΔF508 variant only
Osborne *et al.*	1992	24	Selective screening for the N1303K variant only
Carles *et al.*	1996	25	PCR, DGGE (exon for exon), and finally direct sequencing of aberrant migrating or heteroduplex DGGE bands
Padoa *et al.*	1999	26	Selective screening for four variants, SSCP analysis to detect an additional variant, and sequencing of aberrant bands that indicated the presence of a sixth CF-associated allele. This article states that the ΔF508 variant was not found in this study, but is unclear whether testing for this variant occurred in the CF and suspected CF individuals
Romey *et al.*	1999	27	DGGE and direct sequencing of the minimal CFTR promotor region. Single region studied
Goldman *et al.*	2003	18	In black SA CF patients: selective screening for c.3120+1G>A, and if not found, additionally screened for ΔF508. In coloured SA CF patients: selective screening for 24 variants. Five patients screened only for ΔF508. In white SA CF patients: Selective screening for 24 variants
Des Georges *et al.*	2008	28	SQF-PCR and WGA with MLPA in patients whose samples did not undergo WGA
De Carvalho and Ramsay	2009	29	MLPA for CNV detection
Masekela *et al.*	2013	17	Selective screening for the c.3120+1G>A variant in patients in whom either no variants or only a single variant had previously been detected. Variant data not stratified according to ethnicity and only the c.3120+1G>A variant results reported

CF = cystic fibrosis; SA = South African; PCR = polymerase chain reaction; DGGE = denaturing gradient gel electrophoresis; SSCP = single-strand conformation polymorphism; CFTR = cystic fibrosis transmembrane conductance regulator; SQF = semiquantitative fluorescent multiplex; WGA = whole-genome amplification; MLPA = multiplex ligation-dependent probe amplification; CNV = copy number variation.

programmes.[12,32,33] In 2011, neonatal screening accounted for the detection of 74% of all new CF cases in the UK.[13] This has been highly beneficial, since early detection of CF can reduce negative nutritional outcomes, general health complications and decline in cognitive capacity, thereby improving the overall health of affected patients.[34,35] Early diagnosis has also been shown to provide an economic benefit. For example, Dutch patients identified by newborn screening programmes spend about a million euros less on treatment over the course of their lives than patients not discovered through this method.[36] Interestingly, despite the clearly demonstrated benefit of early diagnosis, in studies conducted on SA CF patients there is no correlation between specific CF variants and the severity of lung function decline and associated nutritional status.[37,38]

Based on CF variant data that are currently available, the most common CF variants in SA are ΔF508, c.3120+1G>A, c.3272-26A>G and 394delTT, which have been identified in 69.8%, 13.2%, 3.4%, and 3.0% of screened patients, respectively (Table 2). However, as reflected in Table 1, different screening methods were used in the detection of these variants. Although not always feasible, especially in early studies, it must also be noted that not a single reported study has made use of an unbiased DNA sequencing approach. For example, many black SA patients are screened only for the presence of the c.3120+1G>A variant in order to make a positive molecular diagnosis of CF.[17,18,39] Novel or SA-specific mutations in the major population groups have therefore gone undiscovered or could not have been detected owing to limited screening capacity. An additional confounding factor, as illustrated in Table 3, is that SA CF data have largely been dominated by white patients, a minority genetic group in the country. Although historical factors are largely responsible for this phenomenon, access to and the cost of sequencing technology now make it possible to obtain new information with relative ease, affordability and speed.[40] Use of advanced sequencing methods, particularly in majority population groups, would be of benefit to SA CF patients, since SA is home to some of the most genetically diverse population groups in the world.[41]

A need for more molecular data to assist with accurately diagnosing SA and African CF patients therefore exists. Nevertheless, as illustrated by existing CF registries, several types of data (and not just causative variant information) are investigated. In the absence of molecular data, developing algorithms that can aid in the diagnosis of CF given limited clinical, biochemical and/or molecular information may be invaluable to SA clinicians in the interim. Although such programmes/tools are only as good as the quality and quantity of available data, they at least have the potential to present baseline probabilities as to a positive CF diagnosis. Such tools might easily serve as a standard by which potential CF patients could be selected for sequencing projects, thereby maximising the odds of identifying previously undescribed CF variants. In so doing, more exact data could be fed back into diagnostic algorithms to improve their accuracy.

Conclusions

The lack of standardised SA CF data is having a negative impact on the longevity of SA CF patients. Driving CF research with the distinct aims of: (*i*) identifying CF variants relevant to all SA population groups; (*ii*) establishing and maintaining a country-specific CF database/registry; (*iii*) establishing a solid foundation for a newborn screening programme; and (*iv*) exploring novel means through which a positive clinical diagnosis could be made (given limited molecular data) would be of great benefit to the overall care received by many local patients. The first of these points can be achieved through the use of next-generation sequencing methods, whole-exome sequencing, and/or targeted sequencing of the *CFTR* (cystic fibrosis transmembrane conductance regulator) gene. Although presenting

Table 2. Variant frequencies based on definitive outcomes*

Variant	White	Black	Mixed	Black and mixed	Not stated	Total frequency
ΔF508	78.32 (531/678)	0 (0/52)	50.88 (58/114)	-	-	69.79 (589/844)
3120+1G>A	0.5 (2/402)	31.25 (20/64)	17.44 (15/86)	73.33 (44/60)	-	13.24 (81/612)
3272-26A>G	3.98 (16/402)	0 (0/6)	1.16 (1/86)	-	-	3.44 (17/494)
394delTT	3.73 (15/402)	0 (0/6)	0 (0/86)	-	-	3.04 (15/494)
G542X	1.74 (7/402)	0 (0/6)	2.33 (2/86)	-	-	1.82 (9/494)
c.54-1161_c.164+1603del2875	-	5.56 (1/18)	0 (0/48)	-	-	1.52 (1/66)
G551D	0.75 (3/402)	0 (0/6)	2.33 (2/86)	-	-	1.01 (5/494)
W1282X	1.0 (4/402)	0 (0/6)	0 (0/86)	-	-	0.81 (4/494)
R553X	1.0 (4/402)	0 (0/6)	0 (0/86)	-	-	0.81 (4/494)
N1303K	1.0 (4/402)	0 (0/6)	0 (0/86)	-	0 (0/14)	0.79 (4/508)
94G>T	0 (0/402)	5.56 (2/36)	0 (0/86)	-	-	0.38 (2/524)
D1270N	-	8.33 (2/24)	-	-	-	8.33 (2/24)
G1249E	0 (0/402)	4.17 (1/24)	0 (0/86)	-	-	0.20 (1/512)
2789+5G>A	0.25 (1/402)	0 (0/6)	0 (0/86)	-	-	0.20 (1/494)
3196del54	0 (0/402)	16.67 (1/6)	0 (0/86)	-	-	0.20 (1/494)
3659delC	0.25 (1/402)	0 (0/6)	0 (0/86)	-	-	0.20 (1/494)
1717-1G >A	0.25 (1/402)	0 (0/6)	0 (0/86)	-	-	0.20 (1/494)
621+1G>T	0.25 (1/402)	0 (0/6)	0 (0/86)	-	-	0.20 (1/494)
Q493X	0.25 (1/402)	0 (0/6)	0 (0/86)	-	-	0.20 (1/494)
R1162X	0 (0/402)	0 (0/6)	1.16 (1/86)	-	-	0.20 (1/494)
R117H	0.25 (1/402)	0 (0/6)	0 (0/86)	-	-	0.20 (1/494)
S549N	0.25 (1/402)	0 (0/6)	0 (0/86)	-	-	0.20 (1/494)
2183delAA	0 (0/402)	2.94 (1/34)	0 (0/86)	-	-	0.19 (1/522)
R334W	-	0 (0/6)	-	-	-	0 (0/6)
3849+10kbC>T	-	0 (0/6)	-	-	-	0 (0/6)
A455E	0 (0/402)	0 (0/6)	0 (0/86)	-	-	0 (0/494)
E60X	0 (0/402)	0 (0/6)	0 (0/86)	-	-	0 (0/494)
ΔI507	0 (0/402)	0 (0/6)	0 (0/86)	-	-	0 (0/494)
R347P	0 (0/402)	0 (0/6)	0 (0/86)	-	-	0 (0/494)
S1251N	0 (0/402)	0 (0/6)	0 (0/86)	-	-	0 (0/494)
1078delT	0 (0/402)	0 (0/6)	0 (0/86)	-	-	0 (0/494)
2183AA>G	0 (0/402)	0 (0/6)	0 (0/86)	-	-	0 (0/494)
A559T	-	0 (0/24)	-	-	-	0 (0/24)
S1255X	-	0 (0/24)	-	-	-	0 (0/24)
444delA	-	0 (0/24)	-	-	-	0 (0/24)

*Values before the parentheses indicate variant frequency in %, while values in parentheses indicate allele count/total number of chromosomes sampled. Null values indicate variants that were screened for but not identified, while - indicates unscreened variants. Total frequency is reported regardless of screening method employed. Ethnicities described here are descriptions obtained from associated literature.

their own unique challenges, sincere, multidisciplinary collaborative efforts that involve stakeholders from academic, private, public and government sectors would be able to address the remaining points.[42] Together, these changes could ensure a healthier and longer future for all SA CF patients.

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Conflicts of interest. None.

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Table 3. Summary of available CF variant data derived from diagnosed CF and/or suspected SA CF patients*

Ethnicity†	Chr tested. *n*	ΔF508	3120+1G>A	94G>T	G1249E	N1303K	1078delT	1717-1G >A	2183AA>G	2183delAA	2789+5G>A	3196del54	3272-26A>G	3659delC	394delTT	621+1G>T	c.54-1161_c.164+1603del2875	A455E	Reference
Black	2	?	?	0.5	?	?	?	?	?	?	?	?	?	?	?	?	?	?	27
Black	12	?	0.08	0.08‡	?	?	?	?	?	?	?	?	?	?	?	?	0.08	?	28
Black	6	0	0.67	0	0.17	0	0	0	0	0	0	0.17	0	0	0	0	0	0	25
Black	18	?	0.11	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	26
Black	28	0	0.46	0.04§	0.04¶	-	-	-	-	0.04§	-	0.04¶	-	-	-	-	-	-	18‖
Black	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03**	-	29
Black and mixed	60	-	0.73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17
Mixed	28	0.54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23
Mixed	86	0.50	0.17	0	0	0	0	0	0	0	0	0	0.01	0	0	0	-	0	18‖
Mixed	48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	29
White	114	0.82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23
White	162	0.81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23
White	402	0.76	0.005	0	0	0.01	0	0.002	0	0	0.002	0	0.04	0.002	0.037	0.002	-	0	18‖
Not stated	14	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	24

Ethnicity†	Chr tested. *n*	E60X	ΔI507	G542X	G551D	Q493X	R1162X	R117H	R347P	R553X	S1251N	S549N	W1282X	444delA	A559T	D1270N	S1255X	3849+10kbC>T	R334W	Reference
Black	2	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	27
Black	12	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	28
Black	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25
Black	18	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0.11	0	-	-	26
Black	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18‖
Black	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29
Black and mixed	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17
Mixed	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23
Mixed	86	0	0	0.02	0.02	0	0.01	0	0	0	0	0	0	-	-	-	-	-	-	18‖
Mixed	48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29
White	114	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23
White	162	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23
White	402	0	0	0.02	0.01	0.002	0	0.002	0	0.01	0	0.002	0.01	-	-	-	-	-	-	18‖
Not stated	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24

CF = cystic fibrosis; SA = South African; Chr tested, *n* = number of chromosomes sampled in a study (sample size); CFTR = cystic fibrosis transmembrane conductance regulator.
*Variant frequencies are indicated within the table. Null values indicate variants that were screened for but not identified, - indicates that variant screening was not performed, while ? indicates an inability to discern, from the literature, whether or not variant screening was performed.
†Ethnicity, as reported in each of the described publications, is represented here.
‡Previously reported by Romey *et al.*[27]
§Previously reported (*CFTR* mutation database: http://www.genet.sickkids.on.ca/cftr/app).
¶Previously reported by Carles *et al.*[25]
‖Goldman *et al.*[39] (2001) is not reflected in this table in order to avoid potential duplication of data from those reported in Goldman *et al.*[18] (2003).
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