REVIEW | Genetics inMedicine

Cystic fibrosis on the African continent

Cheryl Stewart, BSc, PhD[1] and Michael S. Pepper, MD, PhD[1]

Cystic fibrosis (CF; OMIM 219700) is a life-shortening and costly autosomal recessive disease that has been most extensively studied in individuals of Caucasian descent. There is ample evidence, however, that it also affects other ethnicities. In Africa there have been several reports of CF, but there has been no concerted effort toward establishing the molecular epidemiology of this disease on the continent, which is the first step toward outlining a public health strategy to effectively address the needs of these patients. A literature search revealed reports from only 12 of the 54 African states on the molecular analysis of the mutations present in suspected CF patients, resulting in the identification of 79 mutations. Based on previous functional investigations, 39 of these cause CF, 10 are of varying clinical consequence, 4 have no associated evidence regarding whether they cause CF, 4 are synonymous, 5 are novel, and 21 are unique to Africa. We propose that CF be more thoroughly investigated on the continent to ensure that the public health needs of African CF patients—both those in Africa and those of African descent living elsewhere—are met.

Genet Med advance online publication 10 December 2015

Key Words: Africa; cystic fibrosis; molecular epidemiology

INTRODUCTION

Overview

Our awareness necessity of cystic fibrosis (CF) significantly predates our ability to comprehend the molecular factors that underpin its cause and affect prognosis. The dire warning "Woe to that child which when kissed on the forehead tastes salty. He is bewitched and soon must die" has been circulating since the 1800s.[1] CF patients taste salty when kissed because an elevated level of sweat chloride is a hallmark of the disease.[2] If untreated, they rarely make it past their first birthday.[3] Typical presentation also includes failure to thrive caused by pancreatic insufficiency and chronic recurrent chest infections.[4]

The molecular cause of CF was not elucidated until 1989, when *CFTR* was identified as the responsible gene.[5] The nearly 2,000 mutations that have since been identified[3] have been grouped into classes. Classes I–III tend to be associated with more severe forms of CF, whereas patients carrying mutations from classes IV–VI tend to have milder symptoms. The possible effects range from the production of functional CFTR with a reduced ability to traffic chloride ions (class VI) to the production of an immature CFTR that is destroyed before it reaches the cell membrane (class I).[3] Drugs are available that target specific mutation classes; other candidates are the subject of research or have progressed to clinical trials.[6]

Diagnosing CF in Africa

The highest reported prevalence of CF is among individuals of Caucasian descent,[7] in whom it is the leading cause of death among autosomal recessive diseases.[7,8] It was assumed that CF only affected Caucasians, which skewed research efforts. A report from the World Health Organization (WHO) published in 2002 revealed that only a few countries, all European, had a mutation detection rate above 95%: Denmark, the Czech Republic, the Netherlands, Belgium, Switzerland, and the Republic of Ireland.[9] North America, along with Australia, New Zealand, Sweden, Portugal, Luxembourg, the United Kingdom, Italy, Malta, and Greece, had mutation detection rates of 80–89%. By contrast, only two African countries had data at the time of the publication of the report: Algeria (60–69% detection rate) and South Africa (70–79%).[9]

CF was first reported in Africa in a black South African baby who died within half an hour of birth.[10] Several years later, twin black African boys became the first confirmed cases of CF at Baragwanath Hospital in Johannesburg, leading the authors to urge clinicians to consider CF as a possible diagnosis in this ethnic group.[11] This recommendation has not always been heeded, to the detriment of non-Caucasian CF patients. Early diagnosis of CF patients is critical because diagnosis after 6 weeks doubles the risk of development of severe pulmonary disease, which is the leading cause of death among CF patients.[12,13]

Several factors prevent the identification of CF patients on the African continent within the critical 6-week window. First, the assumption among clinicians that CF predominantly affects Caucasians has not been completely dispelled, resulting in underdiagnosis among non-Caucasians. Second, CF diagnosis in Africa may be overshadowed by more rampant phenocopic illnesses such as protein energy malnutrition, chronic pulmonary infections, and HIV.[14] Third, CF patients do not always display the classic triad of symptoms; in one study, only 4.6% of CF patients had all three symptoms,[4] increasing the likelihood of misdiagnosis. Fourth, the gold-standard diagnostic for C F—the sweat test[15]—can return false-negative results,

[1]Department of Immunology and Institute for Cellular and Molecular Medicine, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa. Correspondence: Michael S. Pepper (michael.pepper@up.ac.za)

Submitted 26 May 2015; accepted 17 September 2015; advance online publication 10 December 2015. doi:10.1038/gim.2015.157

particularly in mild cases.[16] The sweat test requires technical skill and rigor and can be reliably performed only at larger health centers and hospitals, reducing its availability to rural populations.[17] In Africa, there may still be countries where this test is not available at all. In Sudan, for example, the sweat test was only introduced in 2008 and was available at one hospital in Khartoum.[18] Fifth, almost 2,000 mutations have been identified in CF patients,[3] yet the full complement of causative mutations, particularly in non-Caucasian populations, remains unknown. This eliminates the possibility of relying solely on an existing genetic test for the unambiguous diagnosis of CF.

Determining the mutations that cause CF is complex. Although the ΔF508 mutation has been estimated to account for 70–90% of all the affected CF chromosomes globally,[3,19] common variants are rare. Of the almost 2,000 variants identified, only 20 of them have an individual global frequency in excess of 0.1%.[3] This means that 10–30% of the chromosomes present in CF patients can be expected to carry rare mutations. CF mutations are also population-specific, varying by race and country.[20] The population-specific nature of the causative mutations and the fact that many of these variants are rare complicate the development of comprehensive molecular diagnostic tools. A diagnostic test can be effective only after members of the subpopulations in a country with symptoms suggestive of CF have had their entire *CFTR* sequenced and the identity and prevalence of each variant have been determined. These data can be used to define a diagnostic instrument with a local mutation detection rate above 90%, which is necessary for the test to be used with confidence. Because there are no mutation hotspots, it would be best to adhere to WHO's recommendation to use this population sequencing strategy in 50–100 putative CF patients in a given population in order to establish a relevant diagnostic tool.[9]

CF patient survival

The life expectancy for a European CF patient is approaching 40 years, which is a marked improvement from the life expectancy in 1938 of 6 months.[3] European patients benefit from the fact that research has been conducted on their populations for a longer period, resulting in a higher mutation detection rate. This advantage is frequently enhanced by a newborn screening program (NBSP) that enables early diagnosis. Babies with CF can then commence treatment, which restricts CF-related organ damage. European CF patients also tend to benefit from referral to multidisciplinary CF centers, which have a range of CF professional staff, including pulmonologists, pharmacists, psychologists, social workers, and microbiologists.[21,22] This specialized holistic approach leads to fewer hospitalizations and allows these children to lead healthier lives. CF patient registries are invaluable for accurately determining disease epidemiology. In Europe, for instance, registry data have been used to inform each country's specific CF health policy. It also allows clinicians and researchers to track the impact of various interventions on patient quality of life and life expectancy. It has likewise been useful in identifying the European centers that are the most successful in treating CF.[23–25] All of these data can be used to make the necessary improvements to ensure a high standard of treatment for CF patients.

Data from North America and Australia reflect similar trends in survival and treatment of CF patients. Americans have access to a network of accredited CF care centers.[26] By 2009, all 50 US states had implemented a CF NBSP; as a result, 57.5% of Americans with CF were diagnosed as neonates in 2010. The age at diagnosis decreased from 6 months to 1 month,[27] which positively impacts prognosis.[12,13] If mortality rates among the US CF population remain as they were in 2010, then American CF patients born in 2010 will live to at least 39 years. If mortality rates continue to decrease as they did in the first decade of the millennium, then American CF babies born in 2010 can be expected to live to age 45 years.[27] In Canada, which also has a network of specialized CF centers, median survival for a CF patient based on 2007 data was 49.7 years. Canada is in the process of introducing an NBSP; it is available in approximately 60% of its provinces.[28] A national CF NBSP commenced in 2003 in Australia,[29] where the median age at death from CF is 30 years.[30]

In stark contrast, the life expectancy for a South African CF patient is 20.5 years.[31] There is no South African NBSP or a population-specific genetic test. Additionally, among the North African countries, only Egypt has an NBSP, but it only targets congenital hypothyroidism.[32] An Internet search did not identify any African CF registries. This negatively impacts life expectancy, hospitalization frequency, and the quality of life of Africans with CF.

The maximum annual treatment cost in South Africa per patient has been estimated to be R360,000,[33] which equates to approximately €26,905. This is triple the average annual wage.[34] Based on European and American data, the cost of CF (including indirect costs) can vary from €16,307 to €394,518 per patient,[35] depending on disease severity. Using Australian CF registry data, it was determined that the annual cost per patient with mild CF was €8,736; moderate and severe CF cost €22,071 and €28,994, respectively.[36] The estimated South African cost may reflect the impact of delayed diagnosis on treatment costs—CF patients are, on average, diagnosed at age 13 months.[37] Delayed diagnosis has been shown to have a negative impact on nutrition, growth, and lung function. CF patients who receive a delayed diagnosis also tend to be hospitalized more frequently, with more severe pulmonary exacerbations, increasing treatment costs.[13]

Although it has been established that CF does indeed affect Africans, no comprehensive public health policy exists to increase the longevity of African CF patients. It may be assumed that the incidence of CF is low in relation to the major challenges faced by African health care, such as HIV and tuberculosis. However, without national CF registries and population screening for CF, there is no way to accurately determine the scale of the problem. Additionally, CF is often misdiagnosed, in part because of the prevalence of phenocopic illnesses but also because the mutations that cause CF are population-specific,

necessitating the development of local genetic tests. Because the available commercial genetic tests have largely been designed based on European data, they are not able to detect mutations specific to a given African nation. In the absence of an appropriate public health response, African CF patients are frequently misdiagnosed, allowing organ damage to proceed unchecked. Misdiagnosis thus drives up the cost of the illness while simultaneously decreasing life expectancy. This is in contrast to Europe, where there is a comprehensive continental public health framework for treating CF, with a concomitant improvement in the quality of life and longevity. It was therefore our aim to survey the literature to assess Africa's state of readiness to implement the kind of public health program that would make a significant difference in the quality of life of African CF patients.

MATERIALS AND METHODS

A survey was conducted of the molecular epidemiology of CF in Africa up to 22 January 2015. Google Scholar was used to search for the term "cystic fibrosis," and the name of each of the 49 states that make up continental Africa was used together with the Republic of Cabo Verde, São Tomé and Príncipe, the Republic of Seychelles, the Union of Comoros, and the Republic of Madagascar. To be included, the paper had to report the results of molecular screening done either in or outside Africa on African citizens (that is, individuals born in Africa who had migrated or the children born to these persons). We also included the results of Jewish populations of African origin in whom we could expect minimal admixture with the people of their current country of residence. We included patients with congenital bilateral absence of the vas deferens, a CF-related disease. Carrier screening data were excluded. When potential CF patients were screened for only one mutation and a second mutation remained unidentified, this was not counted in the category of "unknown mutation" because it is possible that the second mutation would have been uncovered if a more thorough investigation had been performed. If the report detailed the search for more than one mutation, then any chromosome that remained unidentified was categorized as "unknown mutation" ("U"). Investigations that searched for only one mutation and in which none of the chromosomes were positive for that mutation were excluded. The data were recorded per chromosome (not per patient), and only individuals in whom at least one mutation was identified were included in the collated data. We chose to record the data per chromosome for consistency given that many CF patients are heterozygotes.

RESULTS

Twelve African countries had published a total of 26 reports of molecular investigations into the cause of CF—Morocco,[38–40] Algeria,[41–43] Tunisia,[40,41,44–46] Libya,[47] Egypt,[48–50] Sudan,[18] Rwanda,[51] Senegal,[52] Cameroon,[52,53] Namibia,[54] Zimbabwe,[55] and South Africa,[2,37,55–60]—for a total of 2,344 chromosomes screened. Only two publications met our inclusion criteria while also screening non-CF patients;[39,58] in these instances, we included only the data obtained from the CF patients described in these publications. Because the publications span the period 1990–2014, methods as diverse as single-strand conformation polymorphism and sequencing were used. There was no standard list of mutations for screening, so there were differences between studies (**Supplementary Table S1** online). This article therefore reports the total number of chromosomes tested for each mutation as well as the number of chromosomes found to be positive for a given mutation. The largest cohorts were investigated in North Africa (1,334 chromosomes) and Southern Africa (760 chromosomes; **Supplementary Table S2** online). Sudan, Rwanda, Cameroon, and Zimbabwe were the only countries where ΔF508 either was not included in the genetic screen or was not detected. In the remaining eight countries, 2,052 chromosomes were screened for ΔF508, which was detected at a frequency of 48%; this is lower than the global average of 70–90%.[3,19]

A total of 79 variants were detected in the included reports, of which 39 have been shown empirically to cause CF, 10 are of varying clinical consequence,[61,62] 4 have no associated evidence of pathogenicity, 4 are synonymous, 5 are novel, and 21 are unique to Africa (4 of the 21 were novel). The most frequently detected alleles were ΔF508 (992 chromosomes), 3120+1G>A (83 chromosomes), G542X (58 chromosomes), N1303K (51 chromosomes), W1282X (48 chromosomes), E1104X (41 chromosomes), 711+1G>T (36 chromosomes), 3272-26A>G (17 chromosomes), and 394delTT (15 chromosomes). It should also be noted that for 224 chromosomes, the methods used did not result in the resolution of the patient's molecular CF status. These instances are represented by "U" in **Table 1**.

Approximately half of the papers (14 of 26) did not use kits or mutation-specific screening methods and therefore could identify novel or Africa-specific variants (**Supplementary Table S1** online). These studies generated data for 1,120 (49%) of the chromosomes screened and led to the identification of 21 mutations that are unique to Africa. The rest of the chromosomes were tested using commercially available kits or mutation-specific methods. Only 12 variants were found in more than one country, underscoring the population-specific nature of CF mutations. Nine countries have published data on the *CFTR* of fewer than 100 patients (per WHO's 2002 directive[9]). It should be noted that in 9 of the 12 countries, variants were discovered that have never been reported outside Africa. Tunisia heads the list with seven variants (2766del8, T665S, F1166C, 4268+2T>G, L1043R, 3279delAinsTCT, and 1811+5A>G), followed by Rwanda (A204T, 3041-71A>G, 4575+2G>A, 3272-32T>C), Egypt (1898+3A>C, T665S), South Africa (-94G>T, c. 54-1161_c.164+1603del2875), Senegal (c.4136+1G>A, EX17a-EX18del), Cameroon (Y1109X, 405+4A>G), Sudan (R1102K), and Zimbabwe (c. 54-1161_c.164+1603del2875). Only two of these Africa-specific variants (T665S and c. 54-1161_c.164+1603del2875) occurred in more than one nation. As of this writing, for the majority of African countries (42) there is no published account of a search for *CFTR* mutations in their population. As

Table 1 Summary of mutations reported in African CF patients

Mutation	No. of alleles tested	+ Alleles	Allele frequency (%)	Nationality
ΔF508[a]	2,052	992	48.34	Egyptian (CBAVD), Algerian, Tunisian, Libyan, Moroccan, Namibian, South African
U	1,448	224	15.47	Egyptian, Algerian, Tunisian Jews, Tunisian, Libyan, Moroccan, Senegalese, South African, Rwandan
G542X[a]	1,192	58	4.87	Tunisian, South African
N1303K[a]	1,134	51	4.50	Egyptian, Algerian, Tunisian, Libyan, South African
W1282X[a]	1,118	48	4.29	Tunisian, South African
3120 + 1G>A[a]	724	83	11.46	Rwandan, South African, Zimbabwean
E1104X[a]	712	41	5.76	Algerian, Tunisian, Libyan
711 + 1G>T[a]	710	36	5.07	Algerian, Tunisian, Moroccan
V201M	568	5	0.88	Tunisian
D1270N[b]	560	5	0.89	Tunisian, Moroccan, South African
T665S[a,c]	556	2	0.36	Egyptian, Tunisian
R74W[b]	542	3	0.55	Tunisian, Moroccan
2766del8[c]	540	10	1.85	Tunisian
F1166C[c]	540	1	0.19	Tunisian
G85E[a]	540	6	1.11	Tunisian
L1043R[c]	540	1	0.19	Tunisian
R1066C[a]	540	1	0.19	Tunisian
Y122X[a]	540	1	0.19	Tunisian
3272-26A>G[a]	488	17	3.48	South African
G551D[a]	488	5	1.02	South African
5T[b]	420	27	6.43	Egyptian (CBAVD), Algerian, Tunisian, Moroccan
1717-1G>A[a]	402	1	0.25	South African
2789 + 5G>A[a]	402	1	0.25	South African
3659delC[a]	402	1	0.25	South African
394delTT[a]	402	15	3.73	South African
621 + 1G>T[a]	402	1	0.25	South African
Q493X[a]	402	1	0.25	South African
R1162X[a]	402	1	0.25	South African
R117H[b]	402	1	0.25	South African
R553X[a]	402	4	1.00	South African
S549N[a]	402	1	0.25	South African
11TG[b]	300	4	1.33	Moroccan
12TG[b]	300	4	1.33	Moroccan
1811 + 5A>G[c,d]	136	1	0.74	Tunisian
4016insT[a]	136	1	0.74	Tunisian
4268 + 2T>G[c,d]	136	2	1.47	Tunisian
I1203V	136	2	1.47	Tunisian
R1158X[a]	136	2	1.47	Tunisian
R785X[a]	136	1	0.74	Tunisian
405 + 4A>G[c]	122	1	0.82	Cameroonian
A204T[c,d]	120	1	0.83	Rwandan
c.1001 + 11C>T	120	5	4.17	Rwandan
c.1898 + 152T>A	120	9	7.50	Rwandan
c.2752-15C>G	120	3	2.50	Rwandan
c.3041-71A>G[c]	120	2	1.67	Rwandan
c.3272-32T>C[c]	120	1	0.83	Rwandan
c.4575 + 2G>A[c]	120	1	0.83	Rwandan

[a]Mutations empirically shown to cause CF. [b]Mutations with varying clinical consequence. [c]Mutations unique to Africa. [d]Mutations reported as novel in the original publication. CBAVD, congenital bilateral absence of the vas deferens; CF, cystic fibrosis; N, normal CFTR allele; U, unknown mutations; ?, number of alleles was not reported.

Table 1 Continued on next page

Table 1 Continued

Mutation	No. of alleles tested	+ Alleles	Allele frequency (%)	Nationality
E527E	120	3	2.50	Rwandan
F693L	120	2	1.67	Rwandan
M470V	120	13	10.83	Rwandan
P1290P	120	6	5.00	Rwandan
Q1463Q	120	14	11.67	Rwandan
T854T	120	52	43.33	Rwandan
7T[b]	80	57	71.25	Egyptian (CBAVD)
9T[b]	80	9	11.25	Egyptian (CBAVD)
c.1418delG[a]	74	?		Egyptian
c.2620-15C>G	74	?		Egyptian
c.3718-24G>A[d]	74	?		Egyptian
c.3877G>A[c]	74	?		Egyptian
3849+10kbC>T[a]	34	1	2.94	Moroccan Jews
G1244E[a]	34	1	2.94	Moroccan Jews
G1249E	34	7	20.59	South African
S549R[a]	34	4	11.76	Moroccan Jews
-94G>T[c]	28	1	3.57	South African
2183delAA[a]	28	1	3.57	South African
3196del54[a]	34	2	5.88	South African
1812-1G>A[a]	20	1	5.00	Algerian
c.1670delC[c]	20	2	10.00	Libyan
1898+3A>C[c]	16	1	6.25	Egyptian
405+1G>A[a]	12	8	66.67	Tunisian Jews
D579G[b]	6	1	16.67	Sudanese
R1102K[c]	6	1	16.67	Sudanese
3729delAinsTCT[c,d]	4	2	50.00	Tunisian
c.54-1161_c.164+1603del2875[c]	4	2	50.00	South African, Zimbabwean
N	4	1	25.00	Tunisian
1609delCA[a]	2	2	100.00	Algerian
c.4136+1G>A	2	1	50.00	Senegalese
EX17a-EX18del[c]	2	2	100.00	Senegalese
R1070W[b]	2	1	50.00	Moroccan
Y1109X	2	2	100.00	Cameroonian

[a]Mutations empirically shown to cause CF. [b]Mutations with varying clinical consequence. [c]Mutations unique to Africa. [d]Mutations reported as novel in the original publication.
CBAVD, congenital bilateral absence of the vas deferens; CF, cystic fibrosis; N, normal CFTR allele; U, unknown mutations; ?, number of alleles was not reported.

shown in **Figure 1**, most of the molecular work has been done in the Northern African populations of Morocco, Algeria, Tunisia, Libya, and Egypt as well as in South Africa. In East Africa, only Rwanda has any published work, and West Africa is represented by Senegal and Cameroon. In Southern Africa, published molecular reports are from Zimbabwe, Namibia, and South Africa.

It should be noted that all but two of the studies[48,49] that could identify novel mutations used techniques other than, or in addition to, sequencing. Most investigations utilized less sensitive methods (e.g., polymerase chain reaction–restriction fragment-length polymorphism (PCR-RFLP); **Supplementary Table S1** online) and progressed to sequencing only if there was an abnormal pattern. In some instances, the authors chose to screen for a specific mutation (such as ΔF508), and if no mutations were detected they proceeded to a different screening method whose result would again determine whether sequencing would be done. In others, if the mutation-specific test yielded no results, then the authors proceeded directly to sequencing. This was one way to address the cost and complexity of sequencing a large gene (*CFTR* is 189 kb long), but it raises the question of what mutations may have been missed via this strategy.

DISCUSSION

CF patients on the African continent have a life expectancy of 20.5 years,[31] whereas in developed countries CF patients have a life expectancy upward of 40 years.[3,26–28] A public health

intervention could be useful in raising African CF patients' life expectancy, with a concomitant improvement in their quality of life. As an example, in 2002, South African life expectancy, including the impact of HIV, was 54.68 years; in 2014, the life expectancy had increased to 62.5 years. This marked improvement is due, in large part, to a concerted public health thrust to ensure the availability of antiretroviral drugs and to reduce mother-to-child transmission of the virus.[63] Similarly, a public health intervention could radically improve the quality of life of Africans with CF. However, our survey has revealed that there is much to be done before such a strategy could be implemented.

One of the issues that needs to be addressed is the lack of epidemiological data. We recommend that each African nation establish a CF registry. This will facilitate accurate determination of the prevalence and incidence of CF in Africa—information that would define its impact and inform each nation's health policy. The registries would also be an invaluable tool for tracking the impact of various interventions on the overall health of CF patients and enable governments to know when they have met or exceeded targets set for improving the life expectancy and quality of life of CF patients.[64]

Diagnosis of CF relies on two standard biochemical analyses: the sweat test and the fecal elastase-1 assay. Both tests should be made available in at least the major medical centers and hospitals. Molecular diagnosis would be an unambiguous way of identifying a CF patient if the full complement of causative mutations was known. However, because the mutations tend to be population-specific, each country will need to determine the mutations present among its people and tailor the molecular diagnostic tools accordingly. Africa is lagging in this regard, with 78% of countries having no published record of any molecular investigation of CF. Three-quarters of the countries that have published molecular screening work appear to have screened fewer than 100 patients, indicating that there are likely to be additional unidentified mutations. In fact, our data indicate that attempts to identify mutations present in *CFTR* failed in approximately 15% of the African chromosomes tested (**Table 1**). A sequencing-based population-specific approach

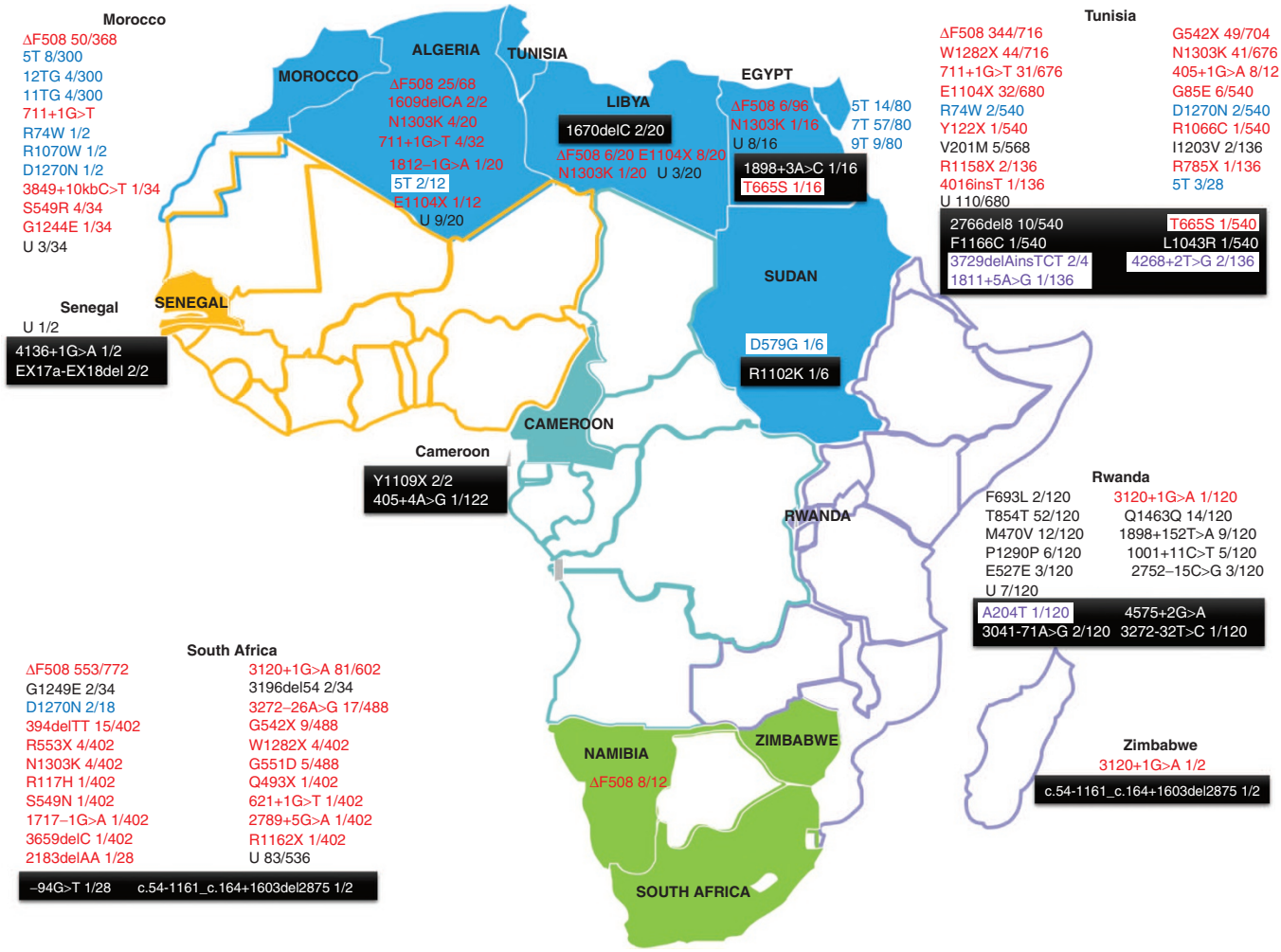

Figure 1 Mutations identified in suspected African cystic fibrosis (CF) patients. Mutations in red have been empirically shown to cause CF, mutations in purple were reported as novel in the original publication, mutations in blue have varying clinical consequence, mutations in black boxes are unique to Africa, and countries in white have no molecular data. The fractions represent the number of each allele identified in a population. The continent was divided into North, West, Central, East, and Southern Africa and color-coded accordingly.

should reduce the percentage of unknown mutations on the continent and provide much needed data for country-specific population tests.

Of the 79 variants identified, 10 are of varying clinical consequence, 5 are synonymous, and 39 are empirically pathogenic. Most of these data were generated as part of the Clinical and Functional Translation of CFTR project (CFTR2), a collaborative initiative that has defined the pathogenicity of variants identified in North American and European CF patients.[61,62] Sixty percent of the variants identified in Africans have been assessed by CFTR2 and thus have also been described in patients of mainly Caucasian descent. This high percentage may first be explained by the fact that in several studies investigators chose to screen for known mutations that had previously been identified in European patients as opposed to taking an unbiased sequencing approach to identifying *CFTR* mutations. Second, it has been shown that there is a certain amount of admixture in North American CF patients, whose population substructures reveal African, Indian, and Mexican influences.[65] Third, there is evidence that Europeans returned to Africa after the initial "out of Africa" migration, leading to European admixture among the peoples of the Mother Continent.[66]

The impact on CFTR of 25 mutations identified in African CF patients still needs to be investigated. The discovery of *CFTR* mutations is insufficient evidence of causation because the identified mutations could be benign. Therefore, functional studies, similar to those described recently,[61] need to be conducted on 32% of the mutations listed in **Table 1** to ascertain how many are deleterious. It is generally accepted that pathogenic mutations reduce CFTR's ion trafficking ability to less than 10%.[61,67] Once the pathogenicity of these variants has been empirically established, these data can be used to develop molecular CF diagnostic tests specific to the subpopulations on the African continent.

It should be noted that for 51% of the chromosomes studied, commercially available kits or mutation-specific screening methods were used. Because these kits were largely developed without African data, they could not identify Africa-specific alleles or novel mutations. To underscore the point, 21 of the 25 mutations that require functional studies have been identified only in Africa. Of these 21, 19 are unique to the particular African nation in which they were identified, lending further credence to the idea that CF mutations are likely to be population-specific. Only 3.8% of the chromosomes were subjected to sequencing without first using some less sensitive molecular screen such as PCR-RFLP. While screening is one way to reduce the cost of sequencing the gene, it also introduces the possibility that some mutations may not have been detected, which is less than ideal.

The abundance of private mutations among African CF patient populations is not unexpected because Africans are known to possess the highest level of genomic variation on the planet and the largest number of population-specific alleles.[68] There are several reasons for this, including the fact that Africa is the birthplace of modern humans, the diversity of the African landscape (which includes deserts and the second largest tropical rainforest in the world), and the selection pressure applied by the high burden of various infectious diseases.[69] Genetic diversity is also indicated by the fact that Africa is home to approximately 2,000 ethnolinguistic groups, which may be taken as a proxy for unique genetic subpopulations because individuals are more likely to reproduce with those who speak their language(s).[70] The participants of the Out of Africa migration experienced a population bottleneck that resulted in the loss of the considerable levels of genomic diversity resident in their African forebears.[68,70]

Despite the unparalleled genomic diversity known to exist among Africans, these populations remain understudied, as has been revealed here with African CF patients. To begin to address this genomic knowledge deficit, the African Genome Variation Project (AGVP) genotyped or performed whole-genome sequencing on 16 populations living in sub-Saharan Africa. The project identified 16–24% novel variants and 11–23% private variants among these subgroups.[66] Based on our survey of variants present in African CF patients, 27% of the mutations identified were private, or unique to the continent. Given the known genetic diversity among the peoples of Africa, it is therefore imperative that African nations develop African screening tools to effectively diagnose and manage African CF patients.

Initially, a complete molecular diagnosis was important only to unambiguously determine a patient's CF status. With recent advances in pharmacotherapy, a molecular diagnosis has also become essential to determining the treatment avenues that might benefit a patient.[6] The approval of Kayledeco by the US Food and Drug Administration (FDA), indicated for the treatment of patients carrying at least one G551D mutation, heralded a new era.[7] Kayledeco is a potentiator that addresses gating defects and may thus be useful in the treatment of other class III mutations. It has been shown to improve conductance in cell lines expressing missense and splicing mutations associated with residual CFTR function.[7] Determining what mutations a CF patient has also means knowing what class(es) those mutations fall into and thus what drug therapies, experimental or approved, may be beneficial. More class-specific drugs are being developed, which means that clinicians would also know which clinical trials a patient may be eligible for. African patients can benefit from both of these scenarios only if the investment is made in diagnosing CF at the molecular level.

It is unfortunately the case that genetics services are seen as luxuries in countries where malnutrition and infection still form a large part of the disease burden. However, there may be a higher prevalence of genetic disorders in developing countries, which tend to underreport these diseases, than in developed nations, making genetic services more relevant in the former. The burden of genetic diseases tends to exact a higher cost there as well, due to limited resources.[71] Having a child with an inherited disease also poses the risk of impoverishing the family and putting their siblings at higher risk of poor health and mortality in countries with a high prevalence

of malnutrition and infection.[72] The WHO therefore says that "Public health authorities must acknowledge the reality that these conditions are indeed major causes of disease, disability, suffering and death in their countries, and recognize that there are approaches for their management and prevention that can significantly reduce their burden in a cost efficient manner" and that "The societal costs of inaction in genetics, measured in terms of avoidable human suffering and burden to public health, are very high." The agency also recognizes that CF belongs to a group of the more common severe genetic diseases and that it could "contribute significantly to chronic morbidity in childhood in many developing countries." It therefore points out that "Programs for the prevention and care of affected children with these conditions may significantly reduce the overall burden due to chronic disease at the community level."[71]

The World Health Assembly (WHA) has recognized that a lack of epidemiological data may prevent effective management of birth defects, defined as any structural or functional abnormality present from birth, such as CF.[73] Based on our survey of CF on the continent, there are no published data concerning the search for the molecular cause of CF in most of the African states. Nor are there registries for CF, which means that there are no reliable baseline epidemiological data (such as incidence and prevalence). Additionally, in the absence of population-specific genetic tests, the true incidence of CF is underreported because there will be an elevated false-negative rate.

The WHA has made several recommendations to improve the treatment of those with birth defects: (i) resources should be devoted to both programs that prevent birth defects and those that provide care for those carrying these defects; (ii) awareness should be raised concerning the importance of newborn screening programs; (iii) data should be collected to accurately determine the scale of the problem; and (iv) appropriate community genetic services should be dispensed within the purview of the primary health-care system.[73] All of these recommendations are relevant to Africa. It is clear from our survey that even with the underreporting there are many CF patients on the continent. A newborn screening program would assist in identifying CF patients soon after birth who could then benefit from starting the appropriate course of treatment earlier, thereby improving their quality of life and longevity. A community genetics approach would allow for both the establishment of national registries and genetic counseling. The former would assist governments in accurately characterizing their CF population and proposing an appropriate public health strategy to improve their overall health. The latter would assist parents of CF patients to understand their child's condition and to make an autonomous decision concerning their future reproductive choices.

By virtue of being born in Africa, CF patients are at a disadvantage and can expect to live only half as long as their European counterparts.[3,31] This disparity is due in large part to the fact that an effective and comprehensive public health strategy to deal with CF does not exist in Africa. This situation must be viewed in the light of Article 24 of the United Nations' Convention on the Rights of the Child, which states that every child should enjoy "the highest attainable standard of health." It calls on States to "strive to ensure that no child is deprived of his or her right of access to such health-care services" via the "application of readily available technology."[74] CF has long been a challenge to health-care systems, particularly among European populations in which the disease prevalence is apparently highest. It is undeniable that CF is also an issue among the people of Africa. However, more epidemiological data need to be gathered in national registries before we can accurately measure the scale of the problem on the Mother Continent. This includes the need to sequence the *CFTR* of suspected CF patients in all the countries that constitute Africa in order to identify the responsible mutations. Given the diversity of Africans, we can reasonably expect that private mutations will be uncovered. Using these molecular data to diagnose patients and commencing treatment as soon as possible would enable fulfillment of the basic health rights afforded to children by the Convention on the Rights of the Child.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

ACKNOWLEDGMENTS

The authors thank the University of Pretoria Vice-Chancellor's Postdoctoral Research Programme, the Institute for Cellular and Molecular Medicine at the University of Pretoria, and the Genomics Research Institute (a University of Pretoria Institutional Research Theme) for funding this study.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Hodson M, Bush A, Geddes D, eds. *Cystic Fibrosis*. 3rd edn. CRC Press: Boca Raton, FL, 2007.
2. Goldman A, Graf C, Ramsay M, Leisegang F, Westwood AT. Molecular diagnosis of cystic fibrosis in South African populations. *S Afr Med J* 2003;93:518–519.
3. Ikpa PT, Bijvelds MJ, de Jonge HR. Cystic fibrosis: toward personalized therapies. *Int J Biochem Cell Biol* 2014;52:192–200.
4. Westwood T, Henderson B, Ramsay M; Medical and Scientific Advisory Committee of the South African Cystic Fibrosis Association. Diagnosing cystic fibrosis in South Africa. *S Afr Med J* 2006;96:304–306.
5. Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989;245:1066–1073.
6. Amin R, Ratjen F. Emerging drugs for cystic fibrosis. *Expert Opin Emerg Drugs* 2014;19:143–155.
7. Bell SC, De Boeck K, Amaral MD. New pharmacological approaches for cystic fibrosis: promises, progress, pitfalls. *Pharmacol Ther* 2015;145:19–34.
8. Snouwaert JN, Brigman KK, Latour AM, et al. An animal model for cystic fibrosis made by gene targeting. *Science* 1992;257:1083–1088.
9. *The Molecular Genetic Epidemiology of Cystic Fibrosis*. World Health Organization: Genoa, Switzerland, 2002.
10. Grove SS. Fibrocystic disease of the pancreas in the Bantu. *S Afr J Lab Clin Med* 1959;5:113–119.
11. Levin SE, Blumberg H, Zamit R, Schmaman A, Wagstaff L. Mucoviscidosis (cystic fibrosis of the pancreas) in Bantu twin neonates. *S Afr Med J* 1967;41:482–485.
12. Wang SS, O'Leary LA, Fitzsimmons SC, Khoury MJ. The impact of early cystic fibrosis diagnosis on pulmonary function in children. *J Pediatr* 2002;141:804–810.

13. Grosse SD, Boyle CA, Botkin JR, et al.; CDC. Newborn screening for cystic fibrosis: evaluation of benefits and risks and recommendations for state newborn screening programs. *MMWR Recomm Rep* 2004;53(RR-13):1–36.
14. Mutesa L, Bours V. Diagnostic challenges of cystic fibrosis in patients of African origin. *J Trop Pediatr* 2009;55:281–286.
15. Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. *Pediatrics* 1959;23:545–549.
16. Highsmith WE, Burch LH, Zhou Z, et al. A novel mutation in the cystic fibrosis gene in patients with pulmonary disease but normal sweat chloride concentrations. *N Engl J Med* 1994;331:974–980.
17. Goldman A, Labrum R, Claustres M, et al. The molecular basis of cystic fibrosis in South Africa. *Clin Genet* 2001;59:37–41.
18. Ibrahim SA, Elmola MAF, Karrar ZA, et al. 2014. Cystic fibrosis in Sudanese children: first report of 35 cases. *Sudan J Pediatr* 2014;14:39–44.
19. Cystic Fibrosis Genetic Analysis Consortium. Population variation of common cystic fibrosis mutations. *Hum Mutat* 1994;4:167–177.
20. Bobadilla JL, Macek M Jr, Fine JP, Farrell PM. Cystic fibrosis: a worldwide analysis of CFTR mutations–correlation with incidence data and application to screening. *Hum Mutat* 2002;19:575–606.
21. Castellani C, Conway S, Smyth AR, Stern M, Elborn JS. Standards of Care for Cystic Fibrosis ten years later. *J Cyst Fibros* 2014;13 Suppl 1:S1–S2.
22. Kerem E, Conway S, Elborn S, Heijerman H; Consensus Committee. Standards of care for patients with cystic fibrosis: a European consensus. *J Cyst Fibros* 2005;4:7–26.
23. Kerem E, Webb AK. European Cystic Fibrosis Society Standards of Care: a road map to improve CF outcome. *J Cyst Fibros* 2014;13:357–358.
24. Stern M, Bertrand DP, Bignamini E, et al. European Cystic Fibrosis Society Standards of Care: Quality Management in cystic fibrosis. *J Cyst Fibros* 2014;13 Suppl 1:S43–S59.
25. Sheppard DN. The European cystic fibrosis patient registry: the power of sharing data. *J Cyst Fibros* 2010;9 Suppl 2:S1–S2.
26. Cystic Fibrosis Foundation—Care Center Network. 2015. http://www.cff.org/LivingWithCF/CareCenterNetwork/. Accessed February 2, 2015.
27. MacKenzie T, Gifford AH, Sabadosa KA, et al. Longevity of patients with cystic fibrosis in 2000 to 2010 and beyond: survival analysis of the Cystic Fibrosis Foundation patient registry. *Ann Intern Med* 2014;161:233–241.
28. Cystic Fibrosis Canada—Annual Report of The Canadian Cystic Fibrosis Registry. 2012. http://www.cysticfibrosis.ca/wp-content/uploads/2014/03/Canadian-CF-Registry-English-FINAL-FOR-WEB1.pdf. Accessed 3 February 2015.
29. Martin B, Schechter MS, Jaffe A, Cooper P, Bell SC, Ranganathan S. Comparison of the US and Australian cystic fibrosis registries: the impact of newborn screening. *Pediatrics* 2012;129:e348–e355.
30. Australian Cystic Fibrosis Data Registry—15th Annual Report from the Australian Cystic Fibrosis Data Registry. 2012. http://www.cysticfibrosis.org.au/media/wysiwyg/CF-Australia/medical-documents/ACFDR_2012/ACFDR_Annual_Report_2012r.pdf. Accessed 3 February 2015.
31. Westwood ATR. The prognosis of cystic fibrosis in South Africa: a 33 year study. *J Cyst Fibros* 2008;7:458.
32. Saadallah AA, Rashed MS. Newborn screening: experiences in the Middle East and North Africa. *J Inherit Metab Dis* 2007;30:482–489.
33. South African Cystic Fibrosis Association—Medication. 2015. http://www.sacfa.org.za/about.asp?r=&spage=9. Accessed 25 July 2014.
34. Statistics South Africa—Census 2011. http://www.statssa.gov.za/publications/P03014/P030142011.pdf. Accessed 25 July 2014.
35. Angelis A, Tordrup D, Kanavos P. Socio-economic burden of rare diseases: A systematic review of cost of illness evidence. *Health Policy* 2015;119:964–979.
36. van Gool K, Norman R, Delatycki MB, Hall J, Massie J. Understanding the costs of care for cystic fibrosis: an analysis by age and health state. *Value Health* 2013;16:345–355.
37. Masekela R, Zampoli M, Westwood AT, et al. Phenotypic expression of the 3120+1G>A mutation in non-Caucasian children with cystic fibrosis in South Africa. *J Cyst Fibros* 2013;12:363–366.
38. de Prada Merino A, Bütschi FN, Bouchardy I, et al. [R74W;R1070W;D1270N]: a new complex allele responsible for cystic fibrosis. *J Cyst Fibros* 2010;9:447–449.
39. Ratbi I, Génin E, Legendre M, et al. Cystic fibrosis carrier frequency and estimated prevalence of the disease in Morocco. *J Cyst Fibros* 2008;7:440–443.
40. Quint A, Lerer I, Sagi M, Abeliovich D. Mutation spectrum in Jewish cystic fibrosis patients in Israel: implication to carrier screening. *Am J Med Genet A* 2005;136:246–248.
41. Boudaya M, Fredj SH, Haj RB, et al. Cystic fibrosis transmembrane conductance regulator mutations and polymorphisms associated with congenital bilateral absence of vas deferens in a restricted group of patients from North Africa. *Ann Hum Biol* 2012;39:76–79.
42. Loumi O, Baghriche M, Delpech M, Kaplan JC, Bienvenu T. Analysis of the complete coding region of the CFTR gene in ten Algerian cystic fibrosis families. *Hum Hered* 1999;49:81–84.
43. Lucotte G, Barré E, Berriche S. Frequency of the cystic fibrosis mutation delta F508 in Algeria. *Hum Genet* 1991;87:759.
44. Hadj Fredj S, Boudaya M, Oueslati S, et al. New frameshift CF mutation 3729delAinsTCT in a Tunisian cystic fibrosis patient. *J Genet* 2013;92:81–83.
45. Fredj SH, Messaoud T, Templin C, des Georges M, Fattoum S, Claustres M. Cystic fibrosis transmembrane conductance regulator mutation spectrum in patients with cystic fibrosis in Tunisia. *Genet Test Mol Biomarkers* 2009;13:577–581.
46. Messaoud T, Fredj SBH, Bibi A, Elion J, Férec C, Fattoum S. Épidémiologie moléculaire de la mucoviscidose en Tunisie. *Ann Biol Clin* 2005;63:s69–70.
47. Hadj Fredj S, Fattoum S, Chabchoub A, Messaoud T. First report of cystic fibrosis mutations in Libyan cystic fibrosis patients. *Ann Hum Biol* 2011;38:561–563.
48. El-Seedy A, Pasquet M-C, Shafiek H, El-Komy M, Kitzis A, Ladevèze V. Cystic fibrosis in Egypt: new mutational detection of the CFTR gene in patients from Alexandria, northern Egypt. *J Cyst Fibros* 2012;12:S53.
49. Naguib ML, Schrijver I, Gardner P, et al. Cystic fibrosis detection in high-risk Egyptian children and CFTR mutation analysis. *J Cyst Fibros* 2007;6:111–116.
50. Lissens W, Mahmoud KZ, El-Gindi E, et al. Molecular analysis of the cystic fibrosis gene reveals a high frequency of the intron 8 splice variant 5T in Egyptian males with congenital bilateral absence of the vas deferens. *Mol Hum Reprod* 1999;5:10–13.
51. Mutesa L, Azad AK, Verhaeghe C, et al. Genetic analysis of Rwandan patients with cystic fibrosis-like symptoms: identification of novel cystic fibrosis transmembrane conductance regulator and epithelial sodium channel gene variants. *Chest* 2009;135:1233–1242.
52. Feuillet-Fieux MN, Ferrec M, Gigarel N, et al. Novel CFTR mutations in black cystic fibrosis patients. *Clin Genet* 2004;65:284–287.
53. Ghanem N, Costes B, Girodon E, Martin J, Fanen P, Goossens M. Identification of eight mutations and three sequence variations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Genomics* 1994;21:434–436.
54. Cystic Fibrosis Genetic Analysis Consortium. Worldwide survey of the ΔF508 mutation—report from the Cystic Fibrosis Genetic Analysis Consortium. *Am J Hum Genet* 1990;47:354–359.
55. des Georges M, Guittard C, Templin C, et al. WGA allows the molecular characterization of a novel large CFTR rearrangement in a black South African cystic fibrosis patient. *J Mol Diagn* 2008;10:544–548.
56. De Carvalho CL, Ramsay M. CFTR structural rearrangements are not a major mutational mechanism in black and coloured southern African patients with cystic fibrosis. *S Afr Med J* 2009;99:724.
57. Westwood T, Brown R. Cystic fibrosis in black patients: Western Cape experiences. *S Afr Med J* 2006;96:288–289.
58. Padoa C, Goldman A, Jenkins T, Ramsay M. Cystic fibrosis carrier frequencies in populations of African origin. *J Med Genet* 1999;36:41–44.
59. Carles S, Desgeorges M, Goldman A, et al. First report of CFTR mutations in black cystic fibrosis patients of southern African origin. *J Med Genet* 1996;33:802–804.
60. Herbert JS, Retief AE. The frequency of the delta F508 mutation in the cystic fibrosis genes of 71 unrelated South African cystic fibrosis patients. *S Afr Med J* 1992;82:13–15.
61. Sosnay PR, Siklosi KR, Van Goor F, et al. Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. *Nat Genet* 2013;45:1160–1167.
62. Aznarez I, Chan EM, Zielenski J, Blencowe BJ, Tsui LC. Characterization of disease-associated mutations affecting an exonic splicing enhancer and two cryptic splice sites in exon 13 of the cystic fibrosis transmembrane conductance regulator gene. *Hum Mol Genet* 2003;12:2031–2040.
63. Statistics South Africa. Mid-year population estimates, 2015. http://www.statssa.gov.za/publications/P0302/P03022015.pdf. Accessed 28 July 2015.
64. *Community Genetics Services*. World Health Organization: Geneva, Switzerland, 2010.
65. Li W, Sun L, Corey M, et al. Understanding the population structure of North American patients with cystic fibrosis. *Clin Genet* 2011;79:136–146.
66. Gurdasani D, Carstensen T, Tekola-Ayele F, et al. The African Genome Variation Project shapes medical genetics in Africa. *Nature* 2015;517:327–332.

67. Ramalho AS, Beck S, Meyer M, Penque D, Cutting GR, Amaral MD. Five percent of normal cystic fibrosis transmembrane conductance regulator mRNA ameliorates the severity of pulmonary disease in cystic fibrosis. *Am J Respir Cell Mol Biol* 2002;27:619–627.
68. Tishkoff SA, Kidd KK. Implications of biogeography of human populations for 'race' and medicine. *Nat Genet* 2004;36(11 suppl):S21–S27.
69. Reed FA, Tishkoff SA. African human diversity, origins and migrations. *Curr Opin Genet Dev* 2006;16:597–605.
70. Campbell MC, Tishkoff SA. African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping. *Annu Rev Genomics Hum Genet* 2008;9:403–433.
71. *Services for the Prevention and Management of Genetic Disorders and Birth Defects in Developing Countries*. World Health Organization: Geneva, Switzerland, 1999.
72. *Community Approaches to the Control of Hereditary Diseases: Report of a WHO Expert Advisory Group*. World Health Organization: Geneva, Switzerland, 1985.
73. *Sixty-Third World Health Assembly WHA63.17: Birth Defects*. World Health Organization: Geneva, Switzerland, 2010.
74. United Nations Convention on the Rights of the Child. http://www.ohchr.org/EN/ProfessionalInterest/Pages/CRC.aspx. Accessed 6 August 2015.