

Cystic fibrosis carrier frequencies in populations of African origin

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Abstract

Cystic fibrosis (CF) is a common autosomal recessive disorder in populations of European descent. However, very little is known about CF in populations of African origin among whom it has been believed to be extremely rare. The aim of this study was to determine if this is the case or whether it is under-reported. A CFTR mutation, 3120+1G→A, which was first reported in three African-American CF patients, has been shown to account for 9-14% of African-American CF chromosomes. It has also been found in 4/6 CF chromosomes in South African blacks and one CF chromosome of Cameroonian origin. In order to determine the carrier frequency of the 3120+1G→A mutation in Africa, 1360 unrelated, healthy subjects were screened. Nine carriers were identified. In addition, two out of five black CF patients with positive sweat tests were found to be heterozygous for the 3120+1G→A mutation and two out of another four black patients with symptoms suggestive of CF, but unconfirmed by sweat tests, were heterozygous for the D1270N mutation. A further three CFTR mutations, A559T, S1255X, and 444delA, which had been found in African-American CF patients, were not identified in the patients or in over 373 healthy subjects tested. The 3120+1G→A mutation has a carrier frequency of 1 in 91 (8/728) in South African blacks with a 95% confidence interval of 1 in 46 to 1 in 197. Since this mutation accounts for between 15% and 65% of CF chromosomes in South African blacks, a corrected CF carrier frequency would be between 1 in 14 and 1 in 59. Hence, the incidence of CF would be predicted to be between 1 in 784 and 1 in 13 924 births in this population. There are several possible reasons why these people are not being detected. Some of these are misdiagnosis as chronic pulmonary infection, malnutrition, tuberculosis, infantile diarrhoea, failure to thrive, or a high infant mortality rate.

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Cystic fibrosis (CF) has been extensively studied in the African-American population where the incidence has been estimated at 1 in 15 000, giving a heterozygote or carrier frequency of 1 in 61.¹ Haplotype and mutation

analysis of the CFTR gene in African-American CF patients has shown a significantly different profile from that observed in white CF patients.² This suggests that admixture alone, estimated at 30% in African-Americans,³ does not account for CF observed in this population group, and that there must therefore be CFTR mutations which originated in the black population. Macek *et al*⁴ found that African-Americans have their own subset of "common" CFTR mutations. Together, eight mutations (405+3A→C, 444delA, G480C, R553X, A559T, 2307insA, 3120+1G→A, and S1255X) account for 23.1% of African-American CF chromosomes. The ΔF508 mutation, presumably introduced by admixture, accounts for a further 48%.⁴

In contrast, there have been very few reports of CF in African blacks with minimal caucasoid admixture. This may be because CF is being underdiagnosed because of confounding diagnoses, such as malnutrition, chronic pulmonary infections, and tuberculosis. The first case of a South African CF child, of Bantu speaking parents, was reported in 1959.⁵ This infant had meconium ileus and died soon after birth. A second case was mentioned in the appendix.⁵ The next case report was of two unrelated Kikuyu infants from Kenya. The female infant was admitted to hospital because of failure to thrive and a respiratory infection. The male child presented with pale, bulky stools and bronchiectasis. A diagnosis of CF was based on positive sweatplate test results.⁶ A few years later, Levin *et al*⁷ described twin Bantu speaking neonates with CF from South Africa; one twin had meconium ileus, while the other had frequent loose stools and respiratory infections. Nearly 30 years passed before the next report of CF in African blacks was published. In 1996, three southern African black CF patients with symptoms typical of those seen in white CF patients, that is, pancreatic insufficiency, respiratory infections, and positive sweat tests, were shown to have CFTR mutations.⁸

The three CFTR mutations identified in the South African black CF patients were 3120+1G→A, G1249E, and 3196del54.⁸ Of these three CF patients investigated, one was homozygous for the 3120+1G→A mutation and the other two were compound heterozygotes, each with the 3120+1G→A mutation on one chromosome and either the G1249E or the 3196del54 mutation on the other chromosome. The 3120+1G→A mutation was first described in three African-American CF patients.⁹ Subsequently, Macek *et al*⁴ found the

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Table 1 Frequency of the 3120+1G→A mutation in healthy black Africans

Chiefdom	No of subjects	No of carriers	Carrier frequency
Southern Africa*	728	8	1 in 91
Nguni	157	0	0
Zulu	57	0	0
Xhosa	52	0	0
Ndebele	23	0	0
Swazi	25	0	0
Sotho/Tswana	372	6	1 in 62
Pedi/Northern Sotho	152	2	1 in 76
Southern Sotho	100	4	1 in 25
Tswana	120	0	0
Tsonga	76	1	1 in 76
Tsonga	53	1	1 in 53
Shangaan	23	0	0
Venda	45	1	1 in 45
Random blacks†	78	0	0
Central Africa	315	1	1 in 315
Central African Republic	218	0	0
Pygmies	83	0	0
Ubangian speakers	135	0	0
Zambia	97	1	1 in 97
West Africa	109	0	0 in 109
Total	1152	9	1 in 128

*All the southern Africans were south eastern Bantu speakers.
†Unknown chiefdom.

3120+1G→A mutation on 18/148 (12.2%) African-American CF chromosomes, indicating that it is a relatively common mutation in this population. If the mutations that have also been found in the white population were excluded, the 3120+1G→A mutation would account for 53% of African-American CFTR mutations.⁴ This mutation has also been found in a black CF patient whose father, the 3120+1G→A carrier, is from Cameroon.⁸ More recently, the mutation has been identified in three Greek families¹⁰ and it has also been shown to be a common CFTR mutation (25% of their CF chromosomes) in Saudi Arabia.¹¹

In an attempt to establish how common CF is in African populations, healthy unrelated subjects were screened for four mutations: 3120+1G→A,⁹ A559T,¹² S1255X,¹³ and 444delA.¹⁴ They occur in African-American CF patients at frequencies of 12.2%, 2.0%, 1.4%, and 0.7%, respectively.⁴

Subjects and methods

DNA samples from 208 San and 1152 unrelated, healthy African blacks from southern, western, and central Africa were studied. The details of the populations are shown in table 1. In response to questionnaires sent out to doctors at respiratory clinics in South Africa, a total of nine blood samples were received from black patients with symptoms suggestive of CF. Information on the patients' parents indicated that there were eight Nguni, four Sotho/Tswana, and six chromosomes of unknown chiefdom. Of the nine patients, five had positive sweat tests, confirming the diagnosis of CF, one patient had a negative sweat test, and the remaining three patients had no sweat test results. Informed consent and, in the case of minors, parental consent was obtained. DNA was extracted using the salting out method.¹⁵

MUTATION DETECTION

The healthy subjects and the nine patients with symptoms suggestive of CF were screened for

four CFTR mutations (3120+1G→A, A559T, 444delA, and S1255X). Mutation detection was performed using PCR amplification followed by restriction endonuclease digestion. Screening methods for the 444delA and S1255X mutations were previously reported by White *et al*¹⁴ and Cutting *et al*,¹³ respectively. The A559T mutation creates an *MseI* site generating fragments of 10, 13, 66, 142, and 194 bp when the mutation is present and 10, 13, 66, and 336 bp fragments in the absence of the mutation. The 3120+1G→A mutation destroys a *BstNI* restriction enzyme site resulting in fragment sizes of 537 and 33 bp in the presence of the mutation and 340, 197, and 33 bp fragments when the mutation is absent.

The nine patients were further investigated by SSCP analysis to detect the G1249E mutation (found in the heterozygous state of one of the original three South African black CF patients)⁸ in exon 20 of the CFTR gene. A positive control was included on each SSCP gel. Subjects showing aberrant bands were sequenced using an ABI 377 automated sequencer. Sequencing showed the presence of the D1270N mutation, the detection of which was confirmed by a PCR assay. The D1270N mutation destroys a *TaqI* restriction endonuclease site.¹⁶ In the absence of the mutation, fragments of 256 and 217 bp are generated. When the mutation is present, a fragment of 473 bp is generated.

All PCR reactions were carried out in a final volume of 25 μ l with 5 pmol of each primer,¹⁷ 125 μ mol/l dNTPs, 1 U *Taq* polymerase, 250 μ mol/l spermidine, and 1 \times *Taq* polymerase reaction buffer. Samples were amplified using the following conditions: 30 cycles of 30 seconds at 94°C, 30 seconds at 53°C, and 45 seconds at 72°C followed by a final extension for 10 minutes at 72°C. The PCR products were digested for two hours and then sized on a 3% HGT agarose gel.

The χ^2 test with Yates's correction and the Fisher exact test were used to calculate whether or not there was any significant statistical difference between chiefdoms.

Results

Of the nine black patients with symptoms suggestive of CF, two were heterozygous for the 3120+1G→A mutation (both had positive sweat tests) and two were heterozygous for the D1270N mutation (a sweat test had not been performed in one and was negative in the other). D1270N was detected by SSCP analysis and subsequent sequencing (of the first patient), while the second chromosome carrying this mutation was identified by means of the PCR assay. The mutations Δ F508, G1249E, A559T, S1255X, and 444delA were not found in any of the patients.

Nine heterozygotes were identified out of the 1152 Bantu speaking, black, non-CF subjects screened for the 3120+1G→A mutation. Details of the number and population groups in which the heterozygotes were found are presented in table 1. In southern African blacks the carrier frequency was 1 in 91 (1.1%) (95% confidence interval (CI) 0.51-2.17%).¹⁸ There

are no significant statistical differences between the carrier frequencies of the mutation when comparing the different chiefdoms in southern Africa. There was also no difference when comparing the carrier frequencies of the populations of southern Africa with central and west African populations and the San. However, when comparing the carrier frequency obtained in the Sotho chiefdom alone (1 in 42) to the other chiefdoms investigated, significant differences were observed between the Sotho chiefdom and the Central African Republic, central Africa, and west Africa.

A subset of the 1152 subjects were tested for a further three mutations. No heterozygotes were identified in 519, 519, and 373 subjects tested for the A559T, S1255X, and 444delA mutations, respectively. With a 95% confidence interval, the frequencies of subjects who are carriers for these mutations are low in African populations (0-0.0074 for A559T and S1255X; 0-0.0092 for 444delA)¹⁸ and the allele frequencies would be even lower. Further screening was, therefore, not warranted.

Discussion

There is now sufficient evidence to confirm that CF is present in African black populations. The question, however, still remains as to how common CF is in Africa, and whether the phenotype observed is significantly different from that seen in populations of European origin. Of the nine black patients suspected of having CF, two were heterozygous for the 3120+1G→A mutation and two were heterozygous for the D1270N mutation. The latter mutation was first described in the heterozygous state in a white male, of English and Italian origin, with clinically diagnosed congenital bilateral absence of the vas deferens (CBAVD).¹⁶ The two patients of this study were both prepubertal males and their vas deferens had not yet been examined. This mutation seems to be very rare as it has only been reported in 1/2083 CF chromosomes.¹⁹ The two patients who are heterozygous for the D1270N mutation may have a milder form of CF as this mutation has been associated with CBAVD and sodium and chloride concentrations in the normal range. The finding of the 3120+1G→A mutation in the two patients, together with the presenting symptoms and positive sweat tests, suggests that they probably have CF.

The 3120+1G→A mutation was initially found on 4/6 (66%) southern African black CF chromosomes.⁸ In order to obtain an accurate carrier frequency for this mutation, of the nine black patients with symptoms suggestive of CF, only the five with positive sweat tests were included. The mutation was found to occur on 2/10 (20%) of these chromosomes. The mutation was thus found on a total of six out of 16 (37.5%; 95% CI 15.2-64.57%)¹⁸ southern African black CF chromosomes. The results in all the southern African black chiefdoms were pooled as there were no statistically significant differences between them and both major linguistic groups were represented among affected subjects. The absence of the common, white, CF causing mutation, ΔF508, and

another three mutations which account for 4.7% of African-American CF chromosomes, indicate that there are other CFTR mutations in the African black population. The identification of these mutations would result in a better estimation of the prevalence of CF in this population.

The overall 3120+1G→A mutation carrier frequency in unaffected African blacks is 1 in 128 (9/1152) (table 1). The 3120+1G→A mutation carrier frequency is higher, but not significantly different, in southern Africa (1 in 91) when compared with the frequency observed in central and west Africa, as well as in the San (1 in 315, 0 in 109, and 0 in 208, respectively).

The mutations A559T, S1255X, and 444delA were not found in the unaffected black subjects screened. These mutations, although occurring at appreciable frequencies in the African-American population, appear to be absent, or rare, in the African black population. The origins of these mutations are uncertain and their increased frequencies in the African-American population may be the result of a founder effect or random drift.

In southern African blacks, the 3120+1G→A mutation carrier frequency is 1 in 91. This is a minimum carrier frequency as it accounts for only 37.5% (15 to 65%) of CF chromosomes in this population. An adjusted carrier frequency can therefore be calculated at 1 in 34 (1 in 14 to 1 in 59) based on a 100% mutation detection. With an annual birth rate of 795 963 (1994/5 figures²⁰), 172 (57-1 015) black CF babies would be expected to be born each year. There are a number of possible reasons why these black CF patients are not being diagnosed. Firstly, because of the common belief that CF is extremely rare in the African population, CF may not be considered as a diagnosis and patients are therefore misdiagnosed. Secondly, infants may be dying before they can be diagnosed with CF. In countries where the infant mortality rate (IMR) is high, the number of deaths from CF may be small in comparison to other causes.

For example, the overall IMR in the South African black population is 52, that is, 52 out of every 1000 live born babies die before the age of 1 year.²¹ It has been estimated that between 1 in 796 and 1 in 13 964 babies is born with CF and therefore only between 0.12 and 2.5% of the IMR would be because of CF. This relatively small proportion could easily be missed. Thirdly, black CF patients may present with milder symptoms (owing to unknown genetic and environment modifying factors) than those seen in the white population. The suggestion of a milder phenotype seems rather unlikely since the five South African black CF patients in whom the 3120+1G→A mutation had been identified (three patients described in Carles *et al*,⁸ two in the present study) and the other three patients with positive sweat tests (described in this study) presented with clinical symptoms typical of those seen in white CF patients.

The 3120+1G→A mutation has recently been reported in Saudi Arabia¹¹ and Greece.¹⁰

Haplotype analysis (using intragenic and extragenic CFTR markers) of 14 unrelated CF patients from four different populations (African-Americans, African blacks, Saudi Arabians, and Greeks), eight CF carriers from southern Africa, and one CF carrier from Cameroon has suggested that the 3120+1G→A mutation is associated with the same haplotype in all four populations (the CF patients and carriers from Africa are those reported in this study).²² This suggests that 3120+1G→A is an old mutation that arose from a single common ancestor, most probably in Africa. The mutation may then have spread to other parts of the world via slave trade routes and hence its presence in north America, Saudi Arabia, and Greece. Other African alleles, like the β globin sickle cell anaemia mutation and the glucose-6-phosphate dehydrogenase deficiency mutation Gd^A, show a similar pattern of spread.^{23 24}

At present the frequency of CF in Africa cannot be accurately determined since many CFTR mutations remain undetected. As more black patients with CF are diagnosed, it will be possible to confirm the true proportion of CF chromosomes carrying the 3120+1G→A mutation in Africa. The detection of other CF causing mutations in this population will provide a more accurate CF carrier frequency and disease prevalence.

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