

of CF-causing mutations it might be prudent to wait until a greater proportion of CF carriers can be detected in the South African CF population, before offering the test. Unless patients are adequately counselled and made aware of the limitations of the test, a great deal of anxiety will be caused.

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The frequency of the delta F508 mutation in the cystic fibrosis genes of 71 unrelated South African cystic fibrosis patients

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Abstract

The common $\Delta F508$ mutation is present in approximately 70% of mutant cystic fibrosis (CF) genes of European and North American populations. The frequency of the $\Delta F508$ mutation has been established for two groups of South African CF subjects. The mutation was found to be present in 82% and 53% of CF genes of white and coloured (i.e. of mixed ancestry) subjects respectively. These findings assist in providing appropriate counselling to individuals who have a family history of CF and in defining laboratory strategies for the establishment of an efficient genetic service for cystic fibrosis.

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Cystic fibrosis (CF) is a severe autosomal recessive disease common in Caucasian populations. The incidence of CF in the white (Caucasian) population of South Africa is approximately 1/12 000 live births; for the coloured population (i.e. people of mixed ancestry) the incidence is approximately 1/1 200 live births.¹ CF is characterised by progressive lung disease

and pancreatic insufficiency. The clinical expression of CF is diverse.

The gene responsible for CF is located on the long arm of human chromosome 7.^{2,3} This gene codes for the cystic fibrosis transmembrane conductance regulator (CFTR). Molecular analysis of CF genes has revealed that there is a common mutation present in approximately 70% of mutant CF genes. This mutation is termed the $\Delta F508$ mutation and is a 3 base pair deletion which results in the loss of a phenylalanine residue at position 508 in the CFTR protein.^{4,5} The frequency with which the $\Delta F508$ mutation occurs varies between different population groups.⁷ Many mutations other than the $\Delta F508$ have been identified in the CF gene. The prevalence of these mutations is low.⁸

Prior to the identification and characterisation of the CF gene, DNA-based diagnosis of CF was performed by linkage studies in CF families with restriction fragment length polymorphisms (RFLPs) linked to the CF locus. This approach is indirect, as a specific mutation is not assayed. Linkage studies have been successfully used to diagnose prenatal CF and determine carrier status within families.⁹ The discovery of identifiable mutations in the CF gene provides the opportunity for directly identifying the mutations causing the disease in specific subjects. The $\Delta F508$ mutation can easily be detected in a mutant CF gene.^{5,10} In practice, the most efficient method of providing a DNA-based diagnostic service for CF is by assaying for the presence of the $\Delta F508$ mutation in CF genes and employing RFLP studies to follow the inheritance of the other mutations. Within the context of a routine diagnostic service it is impractical to search mutant CF genes for mutations other than $\Delta F508$.

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In order to provide a DNA-based diagnostic service for CF, it is necessary to know the frequency with which the $\Delta F508$ mutation occurs in the mutant CF genes of the population to be served. The frequency of the mutation was established for a group of 71 unrelated South African CF subjects from both white and coloured ethnic groups. The genotypes of these subjects were also established.

Subjects and methods

Subjects studied

Seventy-one unrelated subjects diagnosed as having CF were tested for the presence of the $\Delta F508$ mutation in their CF genes. The subjects were divided into two groups according to their ethnic origin. The white group had 57 subjects and the coloured (mixed race) group 14 subjects. The subjects in the coloured group came from the Western Cape; the white subjects were predominantly from Natal and the Western Cape.

DNA analysis

DNA was isolated from peripheral blood samples¹¹ obtained from subjects by venepuncture.

Two methods of detecting $\Delta F508$ were employed. Both methods used the polymerase chain reaction (PCR) for the amplification of the exon 10 gene fragment which includes the $\Delta F508$ mutation. All oligonucleotides used, in both methods, were obtained commercially.

Method 1

The exon 10 gene fragment, including the $\Delta F508$ mutation, was amplified by the PCR. The sequence of primers used for DNA amplification and the allele-specific oligonucleotide (ASO) probes used are those described by Riordan *et al.*³ PCR products were separated by agarose gel electrophoresis and transferred to a membrane by means of the Southern transfer technique. Membranes were hybridised with radio-labelled ASO probes. Autoradiography was used to detect positive hybridisation of ASO probes to amplified DNA. Positive hybridisation of the ASO probe for the normal allele indicated that the $\Delta F508$ mutation was not present. Positive hybridisation of the ASO probe, specific for the $\Delta F508$ mutation, indicated the presence of the mutation. Hybridisation of both ASO probes to amplified DNA from one subject indicated that the subject carried one CF gene with the $\Delta F508$ mutation and one without. A subject with this genotype is heterozygous for the $\Delta F508$ mutation.

Method 2

The PCR was used to amplify the exon 10 gene fragment, including the $\Delta F508$ mutation site, to produce either 50 or 47 base pair products.¹⁰ A 50 base pair product is produced when the $\Delta F508$ mutation is absent from the CF gene and a 47 base pair product when the mutation is present. PCR products were separated by polyacrylamide gel electrophoresis. Analysis was performed after the gels were stained with ethidium bromide.

Subjects who do not carry the $\Delta F508$ mutation in either of their CF genes are identified by the production of only a 50 base pair PCR product. Subjects who are heterozygous for the $\Delta F508$ mutation show both a 50 and a 47 base pair PCR product. Subjects who are homozygous for the $\Delta F508$ mutation show a 47 base pair PCR product only.

Results

The number and proportion (relative to other mutations) of $\Delta F508$ mutations in 142 mutant CF genes are presented in Table I. The subjects studied were grouped according to race. The incidence of the $\Delta F508$ mutation in the white group was found to be 82% and 53% in the coloured population.

TABLE I.
Number and proportion of the $\Delta F508$ mutation in 142 mutant CF genes from 71 cystic fibrosis patients

| Ethnic group | Frequency of mutations in CF genes | | No. of CF genes |
|--------------|------------------------------------|-----------|-----------------|
| | $\Delta F508$ | Other | |
| White | 0,82 (94) | 0,18 (20) | 114 |
| Coloured | 0,53 (15) | 0,47 (13) | 28 |
| Total | 0,77 (109) | 0,23 (33) | 142 |

* 'Other' refers to mutations in CF genes other than $\Delta F508$ mutation. The number of CF genes is indicated in parenthesis.

The analyses performed made it possible to establish the genotypes of the subjects studied. These genotypes were constructed with regard to the presence of the $\Delta F508$ mutation in CF genes. Genotypes of the CF subjects studied are presented in Table II. Sixty-eight per cent of white and 21% of coloured subjects were found to be homozygous for the $\Delta F508$ mutation; 28% of white and 64% of coloured subjects were found to be heterozygous for the mutation. Four per cent of white and 15% of coloured subjects were found not to have the $\Delta F508$ mutation in either of their CF genes.

TABLE II.
Genotypes of 71 cystic fibrosis subjects

| Ethnic group | No. of CF subjects per genotype | | | No. of subjects |
|--------------|---------------------------------|------------------------------|---------------|-----------------|
| | $\Delta F508/\Delta F508$ | $\Delta F508/\text{other}^*$ | Other*/other* | |
| White | 39 (68) | 16 (28) | 2 (4) | 57 |
| Coloured | 3 (21) | 9 (64) | 2 (15) | 14 |
| Total | 42 (59) | 25 (35) | 4 (6) | 71 |

* 'Other' refers to mutations in CF genes other than $\Delta F508$ mutation. Percentages are indicated in parenthesis.

Discussion

The frequency of the $\Delta F508$ mutation in mutant CF genes of white CF subjects is 82%. This figure is consistent with the findings of other studies done on white subjects.⁷ The frequency of the mutation in the coloured group was found to be 53%. Although the coloured group was smaller than the white group, there is a significant difference in the frequency of the $\Delta F508$ mutation in mutant CF genes ($\chi^2 = 10,53$; $0,01 > P < 0,001$).

Without examining the specific mutations present in the CF genes of coloured subjects without $\Delta F508$ mutation, the reasons for the difference in frequency must remain a matter of conjecture. However, the observed difference could be attributed to the size of the coloured sample or to the flow of Caucasian genes into the coloured population.

The genotypes of the CF subjects in both groups provide valuable information for the implementation of a diagnostic service. Each CF subject studied represents a proband of a family who may seek a DNA-based diagnostic test, e.g. prenatal diagnosis of CF. Where the proband is homozygous for the $\Delta F508$ mutation, a direct assay of the mutations causing the disease is pos-

sible. In the group studied, 68% of white families and 21% of coloured families fall into this category. For those families in which the proband is heterozygous for the $\Delta F508$ mutation, the most efficient approach to a molecular diagnosis is to combine the assaying of the $\Delta F508$ mutation with RFLP studies. The RFLP studies establish the inheritance pattern of the non- $\Delta F508$ mutation in the family. In those cases where the proband does not carry a $\Delta F508$ mutation in either mutant CF gene, it is necessary to rely on RFLP studies. Only 6% of all subjects studied fall into the latter category. In order to perform predictive analyses using RFLP studies it is essential to trace the inheritance of CF genes from both parents to an affected child. All three subjects must therefore be available for analysis.

The findings of this study not only assist in defining laboratory strategies for the diagnosis of CF but also provide valuable information for counselling the individual with a positive family history of CF. The carrier status of the members of an extended family can be determined either by direct assay for the $\Delta F508$ mutation, or by the use of RFLP studies for CF mutation tracking. Knowing the carrier status of individuals with a positive family history of CF is important when these individuals are of reproductive age so that appropriate genetic counselling can be provided as to the risk of producing offspring with CF.

Individuals who have a positive family history of CF and are not carriers of a CF mutation can be reassured that there is no risk of producing offspring with CF. The spouse of a CF carrier should be encouraged to have his/her carrier status determined as such couples have an increased risk for offspring with CF. Direct assay of the $\Delta F508$ mutation provides a means of establishing the carrier status of individuals who have no family history of CF. However, the absence of the $\Delta F508$ mutation in both CF genes does not exclude an individual from being a carrier. This is due to the frequency of the $\Delta F508$ mutation in mutant CF genes being less than 100%. The chance of being a carrier is calculated from the theoretical carrier frequency, modified for the specific population group of the individual tested. The modified chance of being a carrier after exclusion of the $\Delta F508$ mutation is presented in Table III.

TABLE III.
Modified chance of an individual being a carrier of a CF mutation after exclusion of the $\Delta F508$ mutation

| Ethnic group | Chance of carrying a CF mutation | |
|--------------|----------------------------------|----------------------------------|
| | Prior to $\Delta F508$ test* | After exclusion of $\Delta F508$ |
| White | 1 in 20 | 1 in 111 |
| Coloured | 1 in 55 | 1 in 117 |

* Theoretical value based on prevalence of CF in specific populations.

The exclusion of the presence of the $\Delta F508$ mutation in the CF genes of the spouse of a known carrier of a CF mutation modifies the risk of the couple's offspring having CF. The modified risks of producing offspring with CF are presented in Table IV.

TABLE IV.
Risk of producing offspring affected with CF by a CF carrier and a spouse for whom the $\Delta F508$ mutation has been excluded

| Ethnic group | Risk of producing CF offspring | |
|--------------|--------------------------------|------------------------------------|
| | Prior to $\Delta F508$ test* | After exclusion of $\Delta F508$ * |
| White | 1 in 92 | 1 in 512 |
| Coloured | 1 in 220 | 1 in 468 |

*Risk calculation: chance of a known carrier passing a mutant CF gene to their offspring, 0.50; multiplied by the carrier frequency or modified carrier frequency for a specific population group; multiplied chance of passing a CF mutant gene to their offspring, 0.50.

The modified chance of being a carrier of a mutation in a CF gene and the risk of producing offspring affected with CF after the exclusion of the $\Delta F508$ mutation are similar for both the white and coloured population groups. This is due to the combined effect of the frequency of the $\Delta F508$ mutation in CF genes and the theoretical carrier frequencies in these two groups.

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