First report of CFTR mutations in black cystic fibrosis patients of southern African origin

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Abstract
Cystic fibrosis (CF) is thought to be rare in the black populations of Africa who have minimal white admixture. Only a few cases have been reported but have not been studied at the molecular level. We report the detection of CFTR mutations in three southern African black patients. One was homozygous for the 3120 + 1G→A mutation, while the other two were compound heterozygotes each with this mutation on one chromosome. The other mutations were G1249E and a previously unreported in frame 54 bp deletion within exon 17a involving nucleotides 3196-3249 (3196del54). The 3120 + 1G→A mutation was first described in American black patients and has been shown to be a common mutation in this population (9-14% of CF chromosomes). It was also found in a black CF patient whose father, the 3120 + 1G→A carrier, is from Cameroon. These data suggest that it is an old mutation which accounts for many of the CFTR mutations in African blacks. (J Med Genet 1996;33:802–804)

Key words: cystic fibrosis; African blacks; CFTR mutations.

Cystic fibrosis (CF) is common in white populations of European descent, with an incidence of about 1 in 2000, but has rarely been described in African populations with minimal white admixture. Among the mixed ancestry population of South Africa, it occurs in roughly 1 in 12 000 live births.1 The incidence of CF in North American blacks has been estimated at 1 in 14 000,2 but the incidence in African blacks without white admixture is unknown. Since 1954, sporadic cases of CF have been reported in African blacks,3–7 and there has been the impression that CF is underdiagnosed because of the high prevalence of confounding diagnoses including malnutrition, chronic pulmonary infections, and, more recently, tuberculosis.

This is the first report of CFTR mutations in black patients with no known white admixture. The chieftoms of the parents are as follows: patient 1, both parents Venda; patient 2, mother Sotho, father Tsawa; patient 3, mother Xhos, father of unknown chieftom. The clinical diagnosis of CF was made in these three cases by repeat positive sweat tests and symptoms compatible and suggestive of CF. None had meconium ileus and all were pancreatic insufficient and had chronic lung infections. All had sputum cultures positive for *Pseudomonas aeruginosa* and two were positive for *Staphylococcus aureus*. By using PCR followed by denaturing gradient gel electrophoresis (DGGE) and sequencing of aberrant migrating or heteroduplex bands, the CFTR gene was scanned exon by exon. The methodology described by Fanen et al and Claustres et al was used with minor modifications. Altered mobilities were observed for PCR products containing exons 16, 17a, and 20 from the three patients (fig 1A). Direct sequencing of the PCR products showed two known CFTR mutations (3120 + 1G→A and G1249E) and a previously unreported mutation (3196del54).

The 3120 + 1G→A mutation in intron 16, a splice site mutation that affects the invariant G at position + 1 of the donor site (Macek et al, in press), was identified in four of the six CFTR genes. The remaining CFTR genes had the G1249E mutation, a missense mutation owing to a G to A transition at nucleotide 3878 in exon 20 and the deletion mutation, 3196del54. The 54 bp deletion affects a region of exon 17a, starting at nucleotide 3196 and predicting the loss of 18 amino acid residues (from codon 1022 to codon 1039) in the 10th transmembrane domain (TM10) of the CFTR protein (fig 1B).

The 3120 + 1G→A mutation, found on 0.67 of CF chromosomes in this study, was first described in three American black patients (Macek et al, in press) and was also found in patient 4, one of two black CF patients in a cohort of 200 CF patients from Montpellier, southern France. This patient's father (the 3120 + 1G→A carrier) is from Cameroon, and his mother (the AF508 carrier) is of mixed ancestry (black, Martinique Island and white, Brittany). CF associated haplotypes were constructed within the three South African families and in the Cameroon family using 11 markers (table 1). The 3120 + 1G→A mutation has been shown to be common in American blacks (14/112 CF chromosomes reported to the CF Genetic Analysis Consortium8) and since this mutation has only been detected in black patients one can assume that it arose in Africa. Since the 3120 + 1G→A mutation has been found in association with the same haplotype in southern Africa and in central west Africa (Cameroon) it is suggested that the
mutation has a single origin and that it arose before the Bantu expansion roughly 2000 years ago. Now that a common black CF mutation has been identified, extensive carrier testing in unaffected people would give an estimate of the true frequency of CF in Africans.

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Table 1: Haplotypes associated with the CFTR mutations in the three South African black patients and one Cameroonian patient

<table>
<thead>
<tr>
<th>Patient</th>
<th>Parental origin</th>
<th>Mutation*</th>
<th>Haplotype†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mother</td>
<td>3120</td>
<td>met XV KM D9 J44 6a 470 1898 2694 3601 3.11</td>
</tr>
<tr>
<td>2</td>
<td>Father</td>
<td>3120</td>
<td>1 1 2 2 1 7 1 2 2 2 2</td>
</tr>
<tr>
<td>3</td>
<td>Mother</td>
<td>3120</td>
<td>1 2 2 1 7 1 2 2 2 2 2</td>
</tr>
<tr>
<td>4</td>
<td>Patient</td>
<td>3196</td>
<td>1 2 2 1 7 1 2 2 2 2 2</td>
</tr>
<tr>
<td>5</td>
<td>Father</td>
<td>3196</td>
<td>1 2 2 1 7 1 2 2 2 2 2</td>
</tr>
</tbody>
</table>

* 3120 = 3120 + 1G→A; 3196 = 3196del54
† Met = MetH/MspI; XV = XV-2c/7aq; KM = KM 19/PstI; D9 = pMP690/MspI; J44 = J44/XbaI; 6a = (gatt), in IVS6a; 470 = M470V in exon 10; 1898 = 1898 + 152T/A; 2694 = 2694TG; 3601 = 3601-65C/A/Hinfl in IVS18; 3.11 = pf3.11/PstI.
‡ Italics show when the phase could not be determined.
§ Patient 4 = Cameroonian CF patient.

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Figure 1: Characterisation of the 3196del54 mutation. (A) Denaturing gradient gel electrophoresis patterns for CFTR exons 16, 17a, and 20. For the mutations 3120 + 1G→A and G1249E, the homoduplex of the mutated allele is situated at a higher position in the gel than the normal homoduplex because of lower stability owing to the replacement of G by A in the mutant. (H: homoduplexes; nh: normal homoduplexes; Mm: mutant homoduplexes). Exon 16: lanes 1 and 2, patient 3 and her mother (heterozygous for 3120 + 1G→A); lane 3, normal sample; lanes 4 and 5, patient 2 and his mother (heterozygous for 3120 + 1G→A); lane 6, patient 1 (homozygous for 3120 + 1G→A). Exon 20: lane 1, patient 2 (heterozygous for G1249E); lane 2, normal control. Exon 17a: lanes 1 and 3, normal controls; lane 2, patient 3 (heterozygous for 3196del54). (B) Sequence context of the 54 bp deletion causing 3196del54. The deleted sequence breakpoints are shown by brackets. For the sequence analysis the mutant band was excited from an agarose gel and the DNA eluted and purified on a SpinX(Costar) column before sequencing.