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# Genetic analysis of Cystic Fibrosis in Pakistan: a Preliminary Report

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## Abstract

**Objective:** Evaluation of the incidence of common SF508 mutation in a cohort of Pakistani children with Cystic Fibrosis (CF).

**Methodology:** The presence of the CFTR gene mutation at position 508 (AF508) was evaluated in 15 consecutively presenting children with CF, proven by clinical features and an unequivocally positive sweat chloride test. The DNA from mononuclear cells was extracted and the AF508 mutation assessed by the amplification refractory mutation system using the polymerase chain reaction.

**Result:** The AF508 mutation was found in 9 cases (60%), of which 5 were homozygous for the disorder.

**Conclusion:** In this preliminary study, the reported frequency of the AF508 mutation in Pakistani children with CF is lower than the reported frequency among the Western Caucasian population. Further studies are needed to characterize the common genetic mutations in Pakistani children with CF (JPMA 50:217, 2000).

## Introduction

Cystic fibrosis is one of the most common autosomal recessive disorders among Caucasian population affecting almost 1 in 2000 population<sup>1</sup>. The disorder is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome<sup>7</sup>, which encodes a protein of 1480 amino acid residues<sup>2</sup>. The mutations in CFTR disrupt the CAMP-regulated chloride channel formed by CFTR and also interfere with its regulation of other ion channels such as the amiloride-sensitive sodium channel and the outwards rectifying chloride channel<sup>3</sup>. Thus specific mutations of the CFTR gene may lead to variable clinical changes and altered phenotype<sup>4-7</sup>. The association of some mutations with severe disease and pancreatic insufficiency is well organized<sup>4,5</sup> and presently over 800 different mutations have been described<sup>8</sup>.

The most common CFTR mutation recognized world wide is a three base pair deletion of the codon for a phenylalanine residue at amino acid position 508 (AF508) in the predicted CF polypeptide<sup>9,10</sup>. Although the relationship of specific mutations with recognizable disease patterns and phenotypes in different populations is well known<sup>11,12</sup> there is little information on the genotypic patterns of CF in Asian populations. This information is important as despite previous considerations, CF has now been reported among Middle Eastern and South Asian subjects<sup>3-16</sup>. In a preliminary study Kabra et al<sup>17</sup> evaluated<sup>13</sup> children with CF in Delhi and found that 54% had the AF508 mutation. There are no published reports of genetic analysis in CF patients from Pakistan. We now report the preliminary results of screening for the AF508 mutation in consecutive 15 children with proven CF from Karachi.

## **Patients and Methods**

A consecutive 15 children (age 3 month - 9 years) with proven cystic fibrosis were included in this study. The diagnosis in all was based on suggestive clinical features and an unequivocally positive sweat chloride test. In all cases the sweat test was performed by pilocarpine iontophoresis on a minimum of 70 g sweat. Chloride concentration of >40 mmol/L was taken as diagnostic of CF12. The clinical features and outcome in this group of patients will be described elsewhere.

### **Extraction of DNA from mononuclear cells**

For mononuclear cell isolation, 5ml blood from each patient was collected in screw cap tubes containing EDTA. An equal volume of saline and blood were evenly mixed and slowly poured on a cushion of histopaque (Sigma, USA). Following centrifugation at 400 X g for 20 min at room temperature, the mononuclear cell layer formed at the junction of histopaque and plasma was removed and transferred to a new tube. After washing once with saline the cells were used for DNA extraction by rapid slating out method<sup>18</sup>. Briefly, the cells were resuspended and homogenized in proteinase K-buffer pH 8.0 (1M NaCl, 0.1 M EDTA, 1% SDS) followed by addition of 10 mg/ml proteinase K to a final concentration of 500 mg/ml. The reaction mixture was kept at 55°C for 2 hours.

Subsequently, the proteins were precipitated by 6 M NaCl and pelleted by centrifugation. The supernatant was transferred to a fresh tube and DNA was precipitated by ethanol, pelleted and dissolved in T.E buffer pH 7.4 (10mM tris, 1mM EDTA).

### **AF508 mutation analysis by Refractory Mutation System (ARMS) Amplification**

All patients were screened for the AF508 mutation using ARMS<sup>19</sup>. A typical ARMS assay comprises two PCRs each conducted using the same substrate DNA. For the identification of a specific mutation, two primers, one complementary to the normal DNA sequence and the other to the mutant DNA were mixed with the target DNA in separate reaction tubes. The standard ARMS reaction consisted of 0.5 ug DNA template, 1 uM of oligonucleotide primers (for sequences: Ferrie et al), 200 uM of each deoxynucleotide triphosphate and 1.5 U Taq Polymerase (advance Biotechnologies, USA) in PCR buffer containing 1.5 mM MgCl<sub>2</sub>, 10 mM Tris pH 8.0 and 50 mM KCl. The amplification was performed for 35 cycles under the following conditions: 94°C denaturation for 2 min, 60°C annealing for 2 min, and 72°C extension for 2 min. A final extension at 72°C was carried out for 10 min. The amplified product was separated in an agarose gel by electrophoresis, visualized by ethidium bromide staining and exposure to UV light. The presence of a 160 base pair specific product denoted a positive result. A positive and a negative control were included in each PCR reaction to monitor efficiency of PCR reaction, presence of PCR inhibitors in the specimen and pipetting errors.

## **Results**

Of the 15 cases evaluated for the presence of AF508 no mutation could be identified. Of the 9 were homozygous for the mutation 6 (Figure).

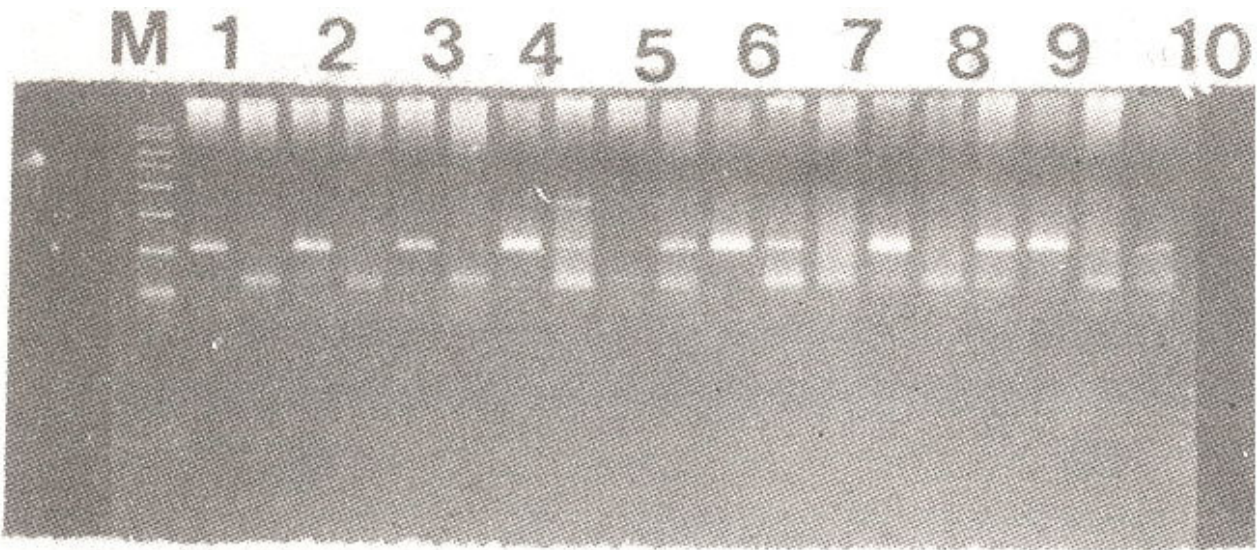


Figure. Results obtained using the standard ARMS test. For each of the 15 samples (lane 1-15) there are two tracks. First lane of each sample contains PCR product of the normal and second lane PCR product obtained with  $\Delta F508$  and control C2 was heterozygous for  $\Delta F508$ . The arrowhead points to 160 bp amplified product.

The overall positivity rate for the AF508 mutation was 60%.

## Discussion

Our preliminary data indicate that only a third of the patients with CF are homozygous for AF508 mutation, which is significantly lower than the reported frequency among the Caucasian population<sup>20</sup>. Previous studies on Pakistani children and families with CF in UK<sup>21,22</sup> and elsewhere<sup>23</sup> have also failed to show the AF508 mutation and a variety of other mutations have been described. A similar study of 216 unrelated patients with CF, including 50 South Indians, reported no cases of AF508 mutation<sup>24</sup>.

Given the diversity of clinical presentation and the relationship of severe disease with specific genotype, it is important to clearly characterise the CF genotype among South Asians. While delayed presentation and diagnosis of CF has been reported previously among Asians<sup>25-27</sup>. Recent data indicates that the disease may also be phenotypically more severe in this ethnic group<sup>16,28</sup>. It is therefore important to characterise the genotype of CF among the south Asian populations. The other important reason is the growing necessity of genotyping in making a diagnosis of CF. Although the sweat chloride test has been widely used to make the definitive diagnosis in suspected patients, there is a well-recognised sub-group with borderline or negative results and the results may be negative in infancy<sup>12</sup>. It may be possible to diagnose CF in infants in buccal cell analysis when quantitative pilocarpine iontophoresis analysis is not possible<sup>29</sup>. Also given the high rates of consanguinity in Pakistan, genotyping of chorion villous sampling may be the only practical option for antenatal diagnosis.

Because of the wide range of mutations in the CFTR gene, unraveling the genotype in CF among Asians is a daunting task. There is a clear need to set up national or regional gene banks and it may be cost-effective to pool resources to do so.

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