Cystic Fibrosis (CF) is one of the most lethal, autosomal, recessive monogenic disorder caused due to an abnormal transport of chloride ions across the apical membranes of epithelial cells.

The sweat glands in CF are relatively impermeable to chloride ions resulting in the increased concentration of chloride in the sweat reaching the skin surface. To maintain electroneutrality, the reabsorption of sodium ions by sweat glands is also reduced, and therefore an increased concentration of sodium also has been observed in the sweat. Thus, a defect in the transport of chloride and sodium ions is characteristic of tissues affected in CF.

However, normal chloride and sodium transport has been observed in some CF cases, indicating the contribution of other pathophysio logic processes. A reduced secretion of bicarbonate ions across the epithelial cells has been reported in some CF cases. Bicarbonate, the body’s major buffer, maintains the alkaline pH in the epithelia, and a reduced bicarbonate secretion, creates an acidic environment leading to the precipitation of mucins and plugging of ductal systems.

The salt or ion movements establish the osmotic driving force for water movement within the tissues. The abnormal ion transport observed in CF, disturbs the hydration state of secretions in the epithelial lumen, due to which the secretions from various glands become dehydrated, thick and sticky, instead of being watery and free-flowing. This eventually leads to plugging of mucous secretions in the ducts of exocrine glands of respiratory, pancreatic, intestinal, biliary and male reproductive tracts.

**CFTR Gene Mutations**

The gene responsible for CF is called the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. It encodes the amino acid product termed the CFTR protein, functioning as a chloride channel. More than 1000 population-specific mutations have been reported in the CFTR gene. These mutations may be mild or severe resulting in partial or complete non-functioning of CFTR chloride channel respectively.

One of the most severe, prevalent and predominant mutation in all the populations is the deletion of phenylalanine at amino acid position 508 (ΔF508) of the CFTR protein.

**Indian Scenario**

CF was considered to be extremely rare in India. The disease was first described in an Indian patient in 1968 from the Post Graduate Institute of Medical Education and Research, Chandigarh. Since then, the published data on Indian CF patients, however, has been very limited. The precise incidence of CF in India is still not known; and the information on CF mutations is also very scarce.

Genetic studies on CF is being carried out at a few research centres in India including P. D. Hinduja National Hospital and Medical Research Centre, Mumbai; All India Institute of Medical Sciences, New Delhi and the Post Graduate Institute of Medical Education and Research, Chandigarh.

The ΔF508 mutation has been reported to exhibit a frequency of approximately 19 - 56% in the Indian CF cases, as compared to 70% in the West. This low frequency may be indicative of the differences in the relative frequencies of CFTR mutations in the Indian CF cases, as compared to the western population.

A screening study of the CFTR gene carried out on Indian CF patients has reported the identification of 2 novel mutations (3622insT, 3601-20T→C/U) and 2 rare mutations (3849+10Kbc→T/U, R560H), along with ΔF508 mutation, thereby anticipating a different spectrum of CF mutations in Indians.

A similar data has been observed during our study carried out at P. D. Hinduja National Hospital and Medical Research Centre, wherein, we attempted at identifying and determining the frequency of 6 of the most common mutations of the world CF population including ΔF508, G542X, G551D, R553X, N1303K and 621+1(g→u) mutations in 23 suspected Indian CF cases, by multiplex ARMS-PCR technique. The ΔF508 mutation, during this study, was observed to possess a frequency of 33%, however, none of the other common mutations were identified.

We then screened the hot spot regions of the CFTR gene (exons 10 and 11) by Single Stranded Conformation Polymorphism / Heteroduplex (SSCP/HD) technique in 139 clinically suspected CF cases. This study, lead to the identification of a rare splice-site mutation (1525-1(G→A) and a common polymorphism (M470V), in addition to the ΔF508 mutation. The 1525-1(G→A) mutation

---

**Abstract**

**Objectives:** CF, caused due to abnormal transport of chloride, sodium and bicarbonate ions across epithelial cell membranes, is a multi-organ disorder. More than 1000 mutations causing CF, have been identified in the CFTR gene, of which AF508 is the most severe, predominant mutation. However, data on CF in India is limited. Also, facilities for CF diagnosis are not available at all diagnostic centres across India.

**Results:** AF508 mutation has been reported in 19 - 56% Indian patients. Also, the spectrum of mutations has been anticipated to be different, due to the identification of a wide range of novel and rare mutations. In addition to mutations, polymorphisms with clinical relevance and practical diagnostic value have also been identified. Clinical profile in Indian patients was also observed to be different.

**Conclusion:** Though, Cystic Fibrosis has always been considered to be a rare disease in India, we hope that the identification of the wide range of mutations, leads us to the recognition of a probable increased incidence of CF in Indian patients. And this would attract greater attention to the diagnosis of this disease, so that a clinically appropriate assay can be developed for their detection as a preliminary test for CF diagnosis. The results observed during the study can be a step forward in planning a molecular screening and providing appropriate genetic counseling programs, which are lacking in our country at the moment.
is predicted to code for a class II defective CFTR protein and hence, classified as a severe pathogenic mutation.11 However, the exact frequency and diagnostic value of this mutation in Indians remains to be established with a larger study population.

We then screened the entire CFTR gene in 96 Indian CF patients in a recent study, by the SSCP analysis, with the aim of identifying all the known as well as unknown mutations. During this study, a total of 14 mutations, including 09 novel [-219insG, -117(G→C), 185+1(G→C), R59X, R75G, 405+1(G→C), S169G, N187D, 3600+6(t→A)], while the remaining mutations were observed to be rare, with most of the mutations being detected in single CF cases (Table 1). In addition, 20 polymorphisms were also identified, of which 7 were novel polymorphisms [-500(A→G), 1342-15(G→T), 4096-265insG, 4096-265insT, L188L, L167L, 622-92(C→A)] and 05 rare, known (S13F, R75Q, 1525-1(G→A), ΔF508, Y569D) mutations were identified in 88 out of 96 CF patients. While, the ΔF508 mutation was found to possess an allele frequency of approximately 53%; the remaining mutations were observed to be rare, with most of the mutations being detected in single CF cases (Table 1). In addition, 20 polymorphisms were also identified, of which 7 were novel polymorphisms [-500(A→G), 1342-15(G→T), 4096-265insG, 4096-265insT, L188L, L167L, 622-92(C→A)] while the remaining 13 [1525-61(A→G), M470V, 1898+152(T→A), T854T, 4005+121delTT, 4521(G→A), 1001+11(C→T), 5/6TGA repeats, 622-100(A→G), 405+46(G→T), 297-67(A→C) and Poly T] were known polymorphisms. Of these, some polymorphisms for e.g., M470V, poly T have been reported to be of clinical relevance and might be of practical diagnostic value.12

Considering the identification of a high percentage of rare and novel mutations, and the ethnic history of Indian population, it may be speculated that the remaining uncharacterized mutations might also not be prevalent mutations, and may prove to be either private mutations or novel mutations. The characteristic CFTR gene mutation distribution pattern in the Indian patients and the mutational heterogeneity observed is in complete agreement with the diverse heterogeneous ethnic origin of the Indian population.

Clinical Presentations in Indian CF Cases

The Indian CF patients mainly present with respiratory and gastrointestinal problems associated with malnutrition. Among these varied clinical symptoms, pulmonary involvement has been observed to be the most predominant and severe CF manifestation.13 This is also in accordance with the observation made during our study, according to which almost 94% of the Indian CF cases exhibit respiratory abnormalities (Table 2).

Although majority of CF cases present during infancy and childhood, a number of cases have been diagnosed in the adulthood. During our study, the cases presenting pancreatic abnormalities especially were observed, to possess a higher age group, indicating that the damage of pancreas inutero occurs progressively; and the patients can present with symptomatic pancreatic abnormalities as the initial manifestations of CF in adulthood.14

According to the literature, CF cases with pancreatic abnormalities exhibits the presence of ΔF508 mutation in the homozygous or heterozygous state.15 However, in our study, majority (>90%) of Indian CF cases with pancreatic abnormalities did not exhibit the presence of ΔF508 mutation, probably suggesting a different clinical profile in the Indian CF cases.10

Clinical severity of CF disease is thus a combination of CFTR mutations influenced by other genetic and environmental factors.16 Marked differences in the clinical severity were observed among genetically identical homozygous ΔF508 cases in the present study, indicating the modifying influence of other genetic factors, such as the presence of a second mutation on the same CFTR allele, attenuating the effect of ΔF508 mutation or the effect of other non-genetic factors such as antibiotic treatment, nutrition, etc. Environmental factors like pollution or tobacco exposure may also play a role.

CF being a recessive disorder, the expression of the disease requires the presence of mutations on both the CFTR alleles. The heterozygous ΔF508 cases may therefore, harbor a second unidentified mutation. However, a single CFTR mutation in combination with specific alleles of other genes or an unfavorable environment can also produce a CF phenotype.17

Table 1: Fourteen CFTR mutations identified in 96 Indian CF patients (Novel mutations)

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Location in CFTR gene</th>
<th>CF alleles (n=192)</th>
<th>% allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>-219insG’</td>
<td>Promoter</td>
<td>5</td>
<td>2.60</td>
</tr>
<tr>
<td>-117(G→C)’</td>
<td>Promoter</td>
<td>2</td>
<td>1.04</td>
</tr>
<tr>
<td>185+1(G→C)’</td>
<td>Intron 1</td>
<td>2</td>
<td>1.04</td>
</tr>
<tr>
<td>R59X*</td>
<td>Exon 3</td>
<td>1</td>
<td>0.52</td>
</tr>
<tr>
<td>R75G*</td>
<td>Exon 3</td>
<td>1</td>
<td>0.52</td>
</tr>
<tr>
<td>405+1(G→C)’</td>
<td>Intron 3</td>
<td>1</td>
<td>0.52</td>
</tr>
<tr>
<td>S169G*</td>
<td>Exon 5</td>
<td>2</td>
<td>1.04</td>
</tr>
<tr>
<td>N187D*</td>
<td>Exon 5</td>
<td>2</td>
<td>1.04</td>
</tr>
<tr>
<td>3600+6(T→C)</td>
<td>Intron 18</td>
<td>1</td>
<td>0.52</td>
</tr>
<tr>
<td>S13F</td>
<td>Exon 1</td>
<td>1</td>
<td>0.52</td>
</tr>
<tr>
<td>R75Q</td>
<td>Exon 3</td>
<td>1</td>
<td>0.52</td>
</tr>
<tr>
<td>1525-1(G→A)</td>
<td>Intron 9</td>
<td>1</td>
<td>0.52</td>
</tr>
<tr>
<td>ΔF508</td>
<td>Exon 10</td>
<td>101</td>
<td>52.6</td>
</tr>
<tr>
<td>Y569D</td>
<td>Exon 12</td>
<td>1</td>
<td>0.52</td>
</tr>
<tr>
<td>Total mutations (14)</td>
<td></td>
<td>122</td>
<td>63.54</td>
</tr>
</tbody>
</table>

Table 2: Clinical presentations observed in Indian CF patients

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory dysfunction</td>
<td>94%</td>
</tr>
<tr>
<td>Nutritional abnormalities</td>
<td>21%</td>
</tr>
<tr>
<td>Pancreatic dysfunction</td>
<td>4%</td>
</tr>
<tr>
<td>Liver abnormalities</td>
<td>3%</td>
</tr>
<tr>
<td>Nasal polyp</td>
<td>1%</td>
</tr>
<tr>
<td>Family history of CF</td>
<td>66%</td>
</tr>
</tbody>
</table>

Majority of the CF patients exhibited multi-organ symptoms

According to the literature, CF cases with pancreatic abnormalities exhibits the presence of ΔF508 mutation in the homozygous or heterozygous state.15 However, in our study, majority (>90%) of Indian CF cases with pancreatic abnormalities did not exhibit the presence of ΔF508 mutation, probably suggesting a different clinical profile in the Indian CF cases.10

Clinical severity of CF disease is thus a combination of CFTR mutations influenced by other genetic and environmental factors.16 Marked differences in the clinical severity were observed among genetically identical homozygous ΔF508 cases in the present study, indicating the modifying influence of other genetic factors, such as the presence of a second mutation on the same CFTR allele, attenuating the effect of ΔF508 mutation or the effect of other non-genetic factors such as antibiotic treatment, nutrition, etc. Environmental factors like pollution or tobacco exposure may also play a role.

CF being a recessive disorder, the expression of the disease requires the presence of mutations on both the CFTR alleles. The heterozygous ΔF508 cases may therefore, harbor a second unidentified mutation. However, a single CFTR mutation in combination with specific alleles of other genes or an unfavorable environment can also produce a CF phenotype.17

CF Diagnosis in India

Variability in the type and severity of CF clinical presentations results in frequent errors of labeling CF with a different diagnosis. The respiratory manifestations mimic those of bronchitis, whooping cough, asthma and chronic lung diseases such as immune deficiency disease, tuberculosis, fungal infections of the lungs, bronchiectasis, etc. The gastrointestinal manifestations mimic those of colic disease, chronic diarrhea and numerous conditions associated with failure to thrive. Thus diagnosis of CF can be easily missed due to a low index of suspicion among the Indians. Also variability in the severity and type of clinical manifestations often lead to delayed diagnosis. Thus, CF may be far more common in people of Indian origin than was previously thought; but is under diagnosed or missed in majority of cases.

The diagnosis of CF is suspected by the presence of a typical CF phenotype and/or family history of CF. CF being a genetic disease, a positive family history should be an important factor in suspecting the disease. However, in view of the recessive mode of inheritance of CF and the present trend towards small families,
the majority of CF cases appear only in a single member of the family, as a result of which the family history does not provide much diagnostic assistance.  

The suspected CF cases are confirmed by the demonstration of a high sweat chloride (>60 mmol/L) concentration. However, sweat testing facilities are not available in most centres in India. The high initial and recurring cost of this test probably makes it less suitable for use in all centres. This poor availability of facilities for CF diagnosis may also be responsible for the under diagnosis and low incidence of CF in India.

A CF patient exhibiting typical CF clinical presentations along with a homozygous or heterozygous ΔF508 mutation may have a normal sweat electrolyte concentration, if a second ameliorating or neutralizing mutation is present elsewhere on the CFTR allele. This suggests that elevated sweat electrolytes may be diagnostic of CF, but a negative sweat test does not completely exclude the possibility of CF.

According to the literature, it is unusual for a child to have a sweat chloride concentration greater than 30-40 mmol/L; and the sweat chloride concentrations increase with age after puberty. Thus, in order to avoid any misdiagnosis, 2 different upper limits (30-40 mmol/L in children and the traditionally used 60 mmol/L in adults) of sweat chloride concentration may be implemented.

The suspected CF cases with a borderline sweat chloride concentration (40-60 mmol/L) presents a diagnostic challenge, as the diagnosis of CF can neither be confirmed nor excluded. In such circumstances, mutation analysis or DNA testing can substitute for the sweat test. The presence of mutations in the CFTR gene can predict with a high degree of certainty that an individual has CF. Also, in some suspected CF cases, especially in infants, the adequate collection of sweat sample becomes difficult and mutation analysis can aid in CF diagnosis, in such circumstances.

Mutation analysis in several populations consists of the screening of a panel of the most common CFTR mutations, so that a mutation detection rate of greater than 90% can be achieved.

The identification of the CF mutations commonly seen in any given population is valuable when mutation analysis is used as a ‘diagnostic test’ for CF. However, since the total number of CF-causing mutations in the Indian patients is likely to be very large, a DNA-based population screening in India will be complicated; and an indirect genetic diagnosis like screening of CF-causing mutations in the Indian patients is likely to be much diagnostic assistance.

Family, as a result of which the family history does not provide the majority of CF cases appear only in a single member of the family, so as a result of which the family history does not provide much diagnostic assistance.

References


Conclusion

Though, Cystic Fibrosis has always been considered to be a rare disease in India, we hope that the identification of the wide range of novel and rare mutations, leads us to the recognition of a probable increased incidence of CF in Indian patients. And this would attract greater attention to the diagnosis of this disease, so that a clinically appropriate assay can be developed for their detection as a preliminary test for CF diagnosis.

Further functional studies will be required to determine whether the molecular mechanisms involved in the base pair substitutions reported in this study could lead to subtle variations in levels of CFTR expression.

The results observed during the study can be a step forward in planning a molecular screening and providing appropriate genetic counseling programs, which are lacking in our country at the moment.

Knowledge about all the mutations will be an essential part in understanding the structure and function of the protein. In conjunction with the development of the three-dimensional structural analysis and tests for the biological activities of CFTR, it will be possible in future, to define the role of all these residues at the molecular level, and eventually, understand the function of the CFTR protein.