Summary:

Cystic fibrosis (also known as CF, mucovoidosis, or mucoviscidosis) is the most common lethal autosomal recessive childhood disorder in Caucasians, occurring in 1/2,500 births (Wilcken et al., 1995 and Massie et al., 2005). In its most common form, CF manifests as progressive lung dysfunction, pancreatic insufficiency, and intestinal disease. Although technically a rare disease, cystic fibrosis is ranked as one of the most widespread life-shortening genetic diseases. It is most common among nations in the Western world; one in twenty-two people of Mediterranean descent is a carrier of one gene for CF, making it the most common genetic disease in these populations.

CFTR gene

The gene which harbors mutations responsible for disease was identified in 1989 (Riordan et al., 1989 and Rommens et al., 1989), after which the protein it encodes was determined to function as a chloride channel that indirectly controls sodium transport. Since then, genetic testing has expanded our understanding of the spectrum of disease that CF represents. The large gene named Cystic Fibrosis Transmembrane Conductance Regulator (the CFTR gene), consists of 27 coding exons. The gene is located on chromosome 7q31.3 having base pairs 116,907,252 to 117,095,950, almost 250,000 bases, encoding 1,480 amino acids (Zielenski et al., 1991).

ΔF508 is the most common mutation with a frequency of 66% worldwide (Zielenski and Tsui, 1995). The frequencies and type of mutations found in populations vary according to the geographic and ethnic origin of the population studied. In Turkish CF patients, 36 mutations accounted for 75% of all CF chromosomes, and 41 CF chromosomes remained unidentified (Kilinc et al., 2002). Accurate knowledge of CF mutations in a specific population provides information for CF prevention programs applicable through heterozygote screening and prenatal diagnosis. Studies performed on Indian patients or patients of Indian origin report 19% to 44% frequency of the ΔF508 mutation (Bowers et al., 1993, Powers et al., 1996 and Kabra et al., 2000); however, the data available on the spectrum of mutations in Indian CF patients is scanty. Haplotype associations have been used to
trace the origin and age of different CF mutations worldwide (Morral et al., 1993). There is no information available in this regard from the Indian subcontinent.

**Pathophysiology of Cystic-fibrosis**

Clinical Aspects: Cystic fibrosis affects the entire body

- Lungs and sinuses
- GI tract, liver and pancreas
- Endocrine system
- Reproductive system

Cystic fibrosis affects epithelial cells in organs where the CFTR protein is found, including lungs, pancreas, intestine, vas deferens, liver, and sweat glands. It is the distribution of the CFTR in these organs that explains much of the multi organ nature of CF. Defects in CFTR function within these organs results in lung disease, pancreatic insufficiency, multifocal biliary cirrhosis, male infertility, and increased sweat ion loss.

**Lungs:** The most serious complications of CF occur in the lungs, due to abnormal epithelial-cell transport of Cl\(^{-}\), resulting in altered surface fluid (Chmiel et al., 2003). The airway surface fluid is decreased due to an increased uptake of sodium (and water), resulting in a dehydrated mucous. These changes impede the necessary ciliary clearance of microorganisms and debris in the lungs, promoting obstruction of the airways and infections. These recurrent infections lead to airway impairment and can cause permanent damage to the lungs (Chmiel et al., 2003). CF patients become infected with specific bacteria, such as *Staphylococcus aureus* or *Haemophilus influenzae*, early in life. As disease progresses, *Pseudomonas aeruginosa* and *Burkholderia* spp may infiltrate the lung (Parameswaran et al., 2007)

**Pancreas:** The exocrine function of the pancreas is responsible for the secretion of enzymes essential for the breakdown of food. In the pancreas, defective CFTR protein causes reduced HCO\(_3\) secretion, leading to congestion of acini and inappropriate activation of pancreatic proteases. This process effectively impairs secretion of the pancreatic enzymes necessary for digestion. Approximately 85% of patients with CF have exocrine pancreas insufficiency, which manifests as poor nutrition and increased fat in stool. This results in weight loss, abdominal pain, and
flatulence (Baker et al., 2005). Replacement of pancreatic enzymes and careful diet planning can overcome many of these problems.

**Liver disease:** While pulmonary and pancreatic disease occurs in 90% of CF patients, liver manifestations occur in no more than one-third of patients. In the hepatic biliary system, CFTR is expressed in cholangiocytes and gall-bladder epithelial cells but not hepatocytes (Cohn et al., 1993). The main role of CFTR within these cells is to regulate the fluid and electrolyte content of bile; its absence or dysfunction results in impaired secretory function, secondary to increased bile viscosity and bile-duct occlusion (Colombo et al., 2007). This stasis results in damage to the hepatocytes and increases pro-inflammatory cytokines, growth factors, and lipid peroxidation.

**Infertility:** Most males with CF are infertile as a result of azoospermia (complete lack of sperm) secondary to the congenital bilateral absence of the vas deferens (CBAVD). In patients with mild disease, infertility may be the first indication that they may have CF. Due to advances in reproductive medicine; spermatozoa can be retrieved in order to overcome the infertility. While 1% to 2% of CBAVD occurs in infertile males without CF, as many as 80% of men with CBAVD have CFTR gene mutations (Khaitov et al., 2005). Infertility in male is also caused by Congenital Unilateral absence of vas deferens (CUAVD).

**Other organ systems:** There are a number of associated morbidities in patients with CF (Figure 1). These manifestations affect the intestine and upper airway, and include sinusitis, nasal polyps, distal ileum obstruction, and meconium ileus.

**Study Design and Subject Selection**

The present work was carried out with the following objectives.

1. Identification of most common ∆F508 mutation in cystic fibrosis patients using allele refractory mutation system (ARMS).
2. Identification and characterization of unknown mutation in CFTR gene from cystic fibrosis patients and their family members using SSCP.
3. To estimate 5T allele and its frequencies.
4. To establish correlation between genotype and phenotype.
We studied 45 patients from the general population in MP, India. Informed consent was provided by all test subjects (Men, Women and children) who were prospectively ascertained over the same time period. Healthy subjects (n = 40) with no signs and symptoms of disease served as controls. Almost 20 unrelated infertile men (14 CBAVD, 6 CUAVD) undergoing fertility treatment (since June 2007 until May 2011), who were diagnosed as having obstructive azoospermia, were selected. Men with CBAVD and CUAVD were ascertained after the diagnosis was confirmed by physical examination, transrectal ultrasound, and evidence of azoospermia / oligospermia. All men with CF had been previously diagnosed by characteristic clinical manifestations and confirmatory diagnostic tests. The patients were 20–50 years of age and were born in MP, India. A child aged 8 years was studied for classical form of Cystic Fibrosis. We also screened patients with chronic pancreatitis (Tropical chronic pancreatitis (TCP), Idiopathic Chronic Pancreatitis (ICP) and Alcoholic chronic pancreatitis (ACP)). Nineteen patients (aged between 20-50 years) with TCP and ACP were finally enrolled for genetic analysis. Informed consent was obtained from the patients.

Following clinical variables of the patients was studied:

1. Age at the time of presentation
2. Sweat chloride concentration as measured by quantitative pilocarpine iontophoresis test.

Most patients with CBAVD (42%) in this study had a mutation in at least one of their CFTR genes, but only one had mutations on both chromosomes. Almost 50% patients with CUAVD had mutation in one chromosome only. Inability to identify the second mutation in most patients with CBAVD, even after all 27 CFTR exons were analyzed, suggests that mutations could be located elsewhere in the non coding regions of CFTR. These mutations may result in a CFTR protein with a normal structure but with low levels of expression (Osborne et al., 1993) which may cause disease only in the organs most sensitive to CFTR dysfunction, such as the vas deferens (Trezise et al., 1993 and Tizzano et al., 1993).

Most common mutation observed in CBAVD and CUAVD was ∆F508 (5 patients), all were heterozygous. N1303K and R117H were the second most common
mutations, followed by G551D and R553X. This is similar to the study of Shastri et al. (2008) which has recommended testing Indian CF patients for ∆F508 followed by 1161delC, 3849+10kbC-T and S549N. In addition, we recommend the inclusion of mutation 1562insA, 1260-1G-A, 3281-3282delC, and c.28G-A in the panel for the genetic diagnosis of CF in India.

A strong association between chronic pancreatitis and CFTR mutations among 19 patients have been detected who were referred for gastrointestinal analysis of pancreatitis. In which 14 patients had single CFTR mutation (73.6 % patients) while none of the patient has two abnormal alleles. Though, 5T allele was present in one patient aged 36 years. 1562insA is the only novel mutation observed in these patients studied, which is reported first time in Indian population. Most common mutation observed was ∆F508 (5 patients), all were heterozygous. One boy, who was suffering from classical form of CF was homozygous for ∆F508.

CF is not uncommon in India and its diagnosis must be done in every suspected patient with abnormal mucous thickening and malabsorption. The spectrum of mutations seems to be heterogenous in the Indian population (Kabra et al., 1998) and the frequency of ∆F508 may be lower than observed in Caucasians. A larger study would be helpful in screening probable mutations in the CFTR gene prevailing in our country.


