Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7, which encodes a protein of 1480 amino acid residues. The CFTR protein functions as a chloride channel regulated by cyclic AMP and controls the regulation of other transport pathways. CF affects epithelial tissue in several organs, causing defective electrolyte transport across the apical membrane, which contributes to the pathogenesis of the disease in the sweat gland, pancreas, intestine, male genital tract, and hepatobiliary system. The traditional diagnostic criteria for CF include persistently elevated concentrations of sweat electrolytes, pulmonary and gastrointestinal disease, male infertility due to obstructive azoospermia, exocrine pancreatic dysfunction, and a family history of cystic fibrosis. The most frequent mutation, present in about 67% of cystic fibrosis chromosomes worldwide, results in the deletion of a phenylalanine residue at codon 508 (delta F508). However, delta F508 is not the predominant mutation in Asian CF patients.

CF is the most common potentially lethal genetic disorder in Caucasian populations, but it is considerably less prevalent in other ethnic groups. It occurs at a frequency of 1 in 2500 in Caucasians, 1 in 15,300 in African Americans, and is very rare in Asian populations. In addition, the different genetic background in Asian patients may have modified the clinical
presentation, such that it may deviate from the classical clinical features of Caucasian CF patients. Current mutation panels are mostly designed to detect $\text{CFTR}$ mutations in Caucasian patients; their detection rate for Asian $\text{CFTR}$ mutation was very low. In the absence of identifiable $\text{CFTR}$ mutations, the diagnosis of CF cannot be confirmed. Thus, a comprehensive, robust mutation screening method is needed to identify the unknown $\text{CFTR}$ mutations in Asians. We recently developed a temporal temperature gradient gel electrophoresis (TTGE) method to screen mutations in Hispanic CF patients. Using this method, 97.5% of Hispanic $\text{CFTR}$ mutations were identified. We applied this method to look for mutations in 2 Taiwanese CF patients with clinical features consistent with CF, but negative results on panethnic mutation panel screening. Novel mutations were identified.

### Patients and Methods

Patient 1 was a Taiwanese boy aged one and a half years (Table). He had elevated sweat chloride of 89 mEq/L, recurrent respiratory tract infection, bronchiectasis, echogenic bowl, developmental delay, and steatorrhea. The patient had a deceased elder sister whose autopsy revealed evidence of CF. However, mutational analysis of the patient’s DNA using the panethnic mutation panel screen (Genzyme Genetics, Framingham, Massachusetts, USA) was negative for 87 recurrent mutations. Because of the positive family history, a comprehensive mutational analysis of the entire $\text{CFTR}$ gene was considered necessary. The whole gene mutation screening by TTGE was performed at the Molecular Genetics Laboratory of the Institute for Molecular and Human Genetics at Georgetown University Medical Center. We have recently described the 2 new mutations in this patient. In this report, we describe the results of molecular studies and carrier testing on the proband and both parents. The patient died before the mutations were eventually identified.

Patient 2 was a 10-year-old Taiwanese girl (Table). CF was diagnosed when she was 6 months of age. She suffered from frequent dehydration with electrolyte imbalance. Sweat chloride was 135 mEq/L. She had numerous episodes of pancreatitis as well as pneumonia and had been treated with pancreatic enzyme and aggressive respiratory care. At age 10, she had physical development in the normal range and was doing well. One of her elder brothers died at age 6 months, due to repetitive respiratory tract infection and pneumonia. The results of detailed pathological studies and clinical history in this patient were reported in 1993. Molecular analysis identified 1 mutation, 1898+5G>T. The other mutant allele was not detected at that time.

### Mutation detection by TTGE

All 27 exons and at least 20 bp of their flanking intron regions were amplified by polymerase chain reaction (PCR) using forward and reverse primers as published previously. Each 50 µL PCR mixture contained 1 X Perkin Elmer PCR buffer (50 mM KCl, 10 mM Tris buffer; pH 8.3), 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.5 µM of each primer, 2.5 units of AmpliTaq DNA polymerase (Perkin Elmer, Applied Biosystem, Foster City, CA), and 100 ng of genomic DNA. The reaction mixture was denatured at 94°C for 4 minutes, followed by 30 cycles of 30 seconds of denaturation at 94°C, 45 seconds of reannealing at optimal temperature and 45 seconds of extension at 72°C. The PCR reaction was completed by a final extension cycle at 72°C for 4 minutes. PCR products were denatured at 95°C for 30 seconds and slowly cooled down to 45°C for a period of 45 minutes at a ramp of 1.1°C/minute. The reannealed homoduplexes and heteroduplexes were kept at 4°C until loading onto the gel. TTGE analysis was performed on a Bio-Rad DCode™ mutation detection system (Bio-Rad laboratories, Hercules, CA). The separation principle of TTGE relies on the differences in the electrophoretic mobility of wild-type and mutant DNA fragments in a linear change of denaturing power achieved by a finely controlled gradual and uniform temperature increase over the
duration of electrophoresis. The temperature range of TTGE for each PCR fragment was determined empirically with the aid of computer simulation (MacMelt, Bio-Rad Laboratories). On TTGE analysis, a homozygous mutation usually shows band shift and a heterozygous mutation shows multiple banding patterns.

**DNA sequencing**

The DNA samples that showed abnormal banding patterns on TTGE analysis, either band shift or multiple bands, were sequenced using the BigDye terminator cycle sequencing kit (Perkin-Elmer, Applied Biosystems) and analyzed on an ABI Prism 377 DNA sequencer (Perkin-Elmer, Applied Biosystems) according to the manufacturer’s protocols. The sequencing data were analyzed using ABI DNA sequencing analysis software (Version 3.0) and compared to GenBank sequence by using Sequence Navigator and DNA Strider.

**Results**

TTGE analysis revealed abnormal banding patterns in 2 exon fragments of Patient 1. Fig. A shows an
abnormal TTGE banding pattern of Patient 1’s exon 1 (lane 4) compared to normal controls in lanes 1 to 3. Sequence analysis revealed a G to T mutation at nucleotide position 151, that changes a glutamate at amino acid residue 7 to a stop codon (Fig. B). Fig. C shows an abnormal TTGE banding pattern of exon 6b. The multiple banding pattern in normal controls (lanes 1 to 3) is due to the heterozygous repeats of (TTGA)\textsubscript{6/7} in intron 6b. Sequence analysis (Fig. D) revealed insertion of an A residue at nucleotide position 989-992. The dublet lower band indicates a heterozygous change. This mutation causes a frameshift and results in a truncated CFTR protein of 306 amino acids. Testing of the parents confirmed their carrier status. The mother carried 989-992insA, and the father carried E7X mutation. Sequence analysis of Patient 2 revealed a total of 3 mutations, 1898+5G>T, 2215insG, and G2816A (S895N) [data not shown].\textsuperscript{15} These 3 mutations have been reported in another unrelated Taiwanese CF family with 2 affected siblings (Patient 6 in Table).\textsuperscript{15} The 2215insG and S895N mutations are cis in the same chromosome.

### Discussion

E7X and 989-992insA mutations have not been reported before, either in Caucasian or other Asian patients. These mutations result in severely truncated CFTR polypeptides that are expected to be of no function. This is consistent with the clinical phenotype of Patient 1, who had meconium ileus and pancreatic insufficiency. With the knowledge of carrier status in both parents provided, genetic counseling and prenatal testing for future pregnancies will become feasible for this family. The incidence of CF in Taiwan is so rare that the patients are often not recognized and/or properly treated. Patient 1 of this report had never been treated with pancreatic enzyme supplement. He died at the age of 18 months. He had an elder sister who had similar clinical features and who also died in early childhood. This case underscores the importance of comprehensive mutational analysis of Asian CF patients, particularly those with positive family history. Identification of mutations will facilitate patient management, carrier testing and genetic counseling. In addition, it is important to document the CFTR mutation spectrum in East Asians so that a mutation panel can be established for effective detection of Asian CF.

We surveyed the literature of reported Chinese/ Taiwanese CF cases and found that only 6 patients, including the 2 patients reported here, had documented molecular analysis (Table). Most residents in Taiwan are immigrants from the southern part of mainland China. Almost all CF patients have pancreatic insufficiency and elevated levels of sweat chloride. The 1898+5G>T is a recurrent mutation in Chinese, which accounts for 4/12 (33%) of Taiwanese/ Chinese CF alleles.\textsuperscript{16} Patients 2 and 6 in Table 1 were not related. Their families lived in different parts of the island for several generations. It is possible that 1898+5G>T and cis 2215insG+S895N are founder CF mutations in Taiwan.\textsuperscript{15} There remains 1 unidentified CFTR mutation in Patient 4 (Table 1). We believe that the 1898+1G>T mutation in Patient 4 is probably of Western origin (Portuguese), and the other unidentified CF allele is of Chinese origin.\textsuperscript{17,18} This is because 1898+1G>T has been found several times in Caucasian populations. It should be noted that the mother of Patient 1 was from Vietnam. It was not clear whether or not she was of Chinese origin. Patient 5 from Shanghai had mutations which were different from the CF mutations in Taiwanese patients. It seems that there is a large pool of novel CF mutations in Chinese which is yet to be identified.

Awareness and recognition of CF in the East is poor. A recent report estimated a CF incidence of 1 in 350,000 Japanese.\textsuperscript{4} Increasing recognition of CFTR mutations as an etiology for differential diagnosis of East Asian patients with diffuse panbronchiolitis, rhinitis, and male infertility\textsuperscript{19,20} will provide the potential to identify the Asian CFTR mutation spectrum and to elevate the quality of patient care. Assuming a CF incidence of 1 in a million Chinese, with a total population of about 1.5 billion in China, the total number of carriers is likely to be over 3 million, a number sufficient to warrant mutation screening.

### References

CFTR Mutations in Taiwanese Patients


