# Genetic Analysis of Ion Transport

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## Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder, and di Sant' Agnese et al. [3] found that the sweat of CF patients contains an excess of sodium and chloride ions. Defects in the regulation of chloride ion transport have been documented in CF epithelial cells [5, 10, 14, 16]. The chloride channel normally responds to $\beta$-adrenergic agents, but CF cells are defective in this response [6, 9, 14]. It has been proposed that the CF defect involves a pathway whereby cAMP regulates ion transport.

The symptoms of CF patients are heterogeneous between and within families [13]. Although most individuals are diagnosed by the time they reach the age of ten, a few remain undiagnosed until adulthood [2, 15]. Approximately 15% of CF patients do not require supplemental pancreatic enzymes and are designated as pancreatic sufficient (PS) [7]. PS is typically concordant within families, suggesting that PS patients may have less severe mutations in the CF gene. However, the heterogeneity within families suggests that additional genetic and environmental factors contribute to the severity of the disease.

The molecular cloning of the CF gene has provided additional research strategies to further understand the disease, and the regulation of ion transport in secretory cells. The gene encodes a 170 kDa polypeptide that is a member of a superfamily of membrane-bound active transport molecules [4, 11, 12]. A three-nucleotide deletion in a putative ATP binding domain has been found in 70% of CF chromosomes; this alteration removes a phenylalanine codon at position 508 ($\Delta$F508). To further understand the relationship between mutations in the gene and the phenotype of patients, we have examined a group of patients who do not contain the common mutation on both chromosomes.

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## Methods and Results

To identify mutations in the CF gene, specific regions were amplified by the polymerase chain reaction (PCR) and assayed for single-stranded conformation polymorphisms (SSCPs). This newly described method allows the rapid screening of samples for the presence of genetic variation [8]. SSCPs are detected by denaturing the DNA and resolving it on nondenaturing acrylamide gels. Each strand of the DNA fragment can potentially form a unique conformation (and



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**Table 1.** Detection of mutations using the SSCP technique

| Exon | PCR product size (bp) | Intron or exon | Number of patients tested | Number of mutation per exon |
|---|---|---|---|---|
| 1 | 180 | E | 150 | |
| 2 | 340 | I | 150 | |
| 3 | 310 | I | 50 | |
| 4 | 210 | E | 150 | 3 |
| 5 | 400 | I | 150 | |
| 6A | 330 | I | 150 | 2 |
| 6A | 420 | I | 150 | |
| 7 | 240 | E | 150 | 3 |
| 8 | 330 | I | 25 | |
| 9 | 560 | I | 150 | |
| 10 | 190 | E | 150 | 1 |
| 11 | 425 | I | 150 | |
| 12 | 420 | I | 75 | |
| 13 | 720 | E | 150 | 2 |
| 14A | 500 | I | 150 | 1 |
| 14B | – | – | – | |
| 15 | 480 | I | – | |
| 16 | – | – | – | |
| 17A | – | – | – | |
| 17B | – | – | – | |
| 18 | 100 | E | 75 | |
| 19 | 250 | E | 150 | 1 |
| 20 | 470 | I | – | |
| 21 | 480 | I | – | |
| 22 | 170 | E | 50 | |
| 23 | – | – | – | |
| 24 | 200 | E | 150 | |

*E:* primers fully contained within the exon; *I:* primers from flanking intron sequence.

have a distinct mobility), and any mutation within that segment can potentially affect the mobility. We screened 150 CF patients who have at least one chromosome that does not contain the common (F508) mutation (Table 1). Primers were chosen to individually amplify coding regions of the gene. Each patient that displayed an aberrantly migrating fragment on an SSCP gel was chosen for the subsequent direct sequence analysis.

Alterations were classified as CF mutations based on the following criteria: (1) The alteration shifts the reading frame and causes premature termination of the protein; (2) An amino acid is replaced with a dissimilar residue, and this alteration does not occur on a large number of normal chromosomes with the same haplotype. Eleven separate CF mutations have been identified, eight of which are frameshift or nonsense mutations and three that replace amino acids (Table 2). Each of the frameshift mutations has been found in only a single family, whereas two of the three-point mutations are found in multiple families. The phenotypes of the patients are summarized in Table 2. All individuals that we have examined that are homozygous for the ΔF508 mutation are pancreatic insufficient (PI) and have moderate to severe disease (data not shown). Most of the patients with the frameshift mutations are homozygous for the absence of the common mutation, and therefore must contain an additional, unidentified mutation. These patients are clinically het-

**Table 2.** CFTR mutations

| Name | Introduced change | Exon | ΔF508[a] status | Number of individuals | Nationality | Pancreatic status |
|---|---|---|---|---|---|---|
| 444delA | Term | 4 | +/+ | 1 | Afr Am | PI |
| G460C | Asp/His | 4 | +/− | 1 | Caucasian | PI |
| G482A | Arg/His | 4 | +/− | 6 | Caucasian | PS |
| 1154insTC | Term | 7 | +/− | 1 | Caucasian | PI |
| G1172C | Arg/Pro | 7 | +/− | 5 | Ger/Fr | PS |
| 1213delT | Term | 7 | +/+ | 1 | It/It | PS |
| 1677delTA | Term | 10 | +/+ | 1 | Russian | − |
| 2522insC | Term | 13 | +/+ | 1 | Italian | PI |
| 2566insAT | Term | 13 | +/+ | 1 | Fr/It | PI |
| C2683T | Term | 14A | +/+ | 1 | Caucasian | PI |
| 3821delT | Term | 19 | +/+ | 1 | Russian | PS |

Mutations are named by nucleotide number, according to Riordan et al. [11].
*CFTR:* CF transmembrane conductance regulator; *Term:* termination;
*Afr Am:* Afro-American; *Ger:* German; *Fr:* French; *It:* Italian.
[a] + refers to wild-type (absence of F508).

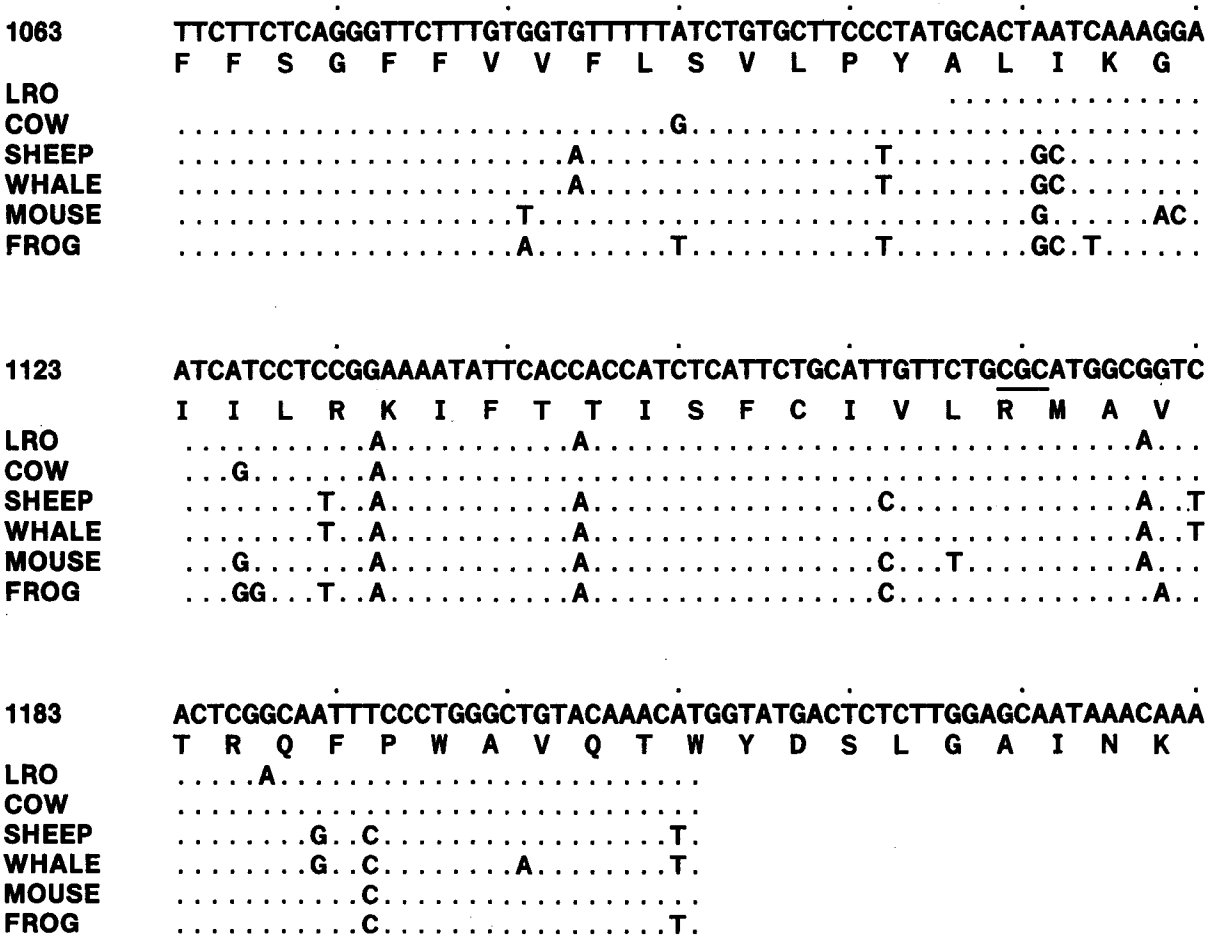


**Fig. 1.** Sequence of a portion of the CFTR transmembrane region from exon 7. The nucleotide sequence is shown from humans, as well as other vertebrate species, with numbering of the human sequence as in Riordan et al. [11]. The *underlined* codon is arginine 347, which was found mutated in several CF patients (mutation G1172C, Table 2). *LRO*: lion tamarin; *WHALE*: humpback whale; *FROG*: *Xenopus*; *MOUSE:* Balb/c

erogeneous; they typically have moderate to severe disease. The majority of the patients with CF point mutations have ΔF508 on their other chromosome, are diagnosed as PS, and have mild disease. Therefore, the type of mutation at the CF locus appears to play an important role in the clinical presentation of the patient.

To further explore the role of the missense mutations in the function of the CF Transmembrane conductance regulator (CFTR) we have amplified the region surrounding these mutations from DNA from a variety of species. The sequence of the species obtained is displayed in Fig. 1. All of the residues that we have found mutated are conserved in all of the species examined. Overall, these regions of the gene show a high degree of conservation, suggesting that alterations in the transmembrane domain are poorly tolerated.

## Discussion

Because the most common mutation accounts for only 70% of CF chromosomes [4], a large proportion of CF patients (40%–50%) are compound heterozygotes, i.e., they have two different mutations in the gene. Thus there is a large number of possible genotypes found in CF patients. This appears to account, in part, for the variation observed in the phenotype of patients. However, within families affected individuals can show differences in sweat chloride levels and severity, demonstrating that additional genetic and/or environmental factors contribute to these phenotypes.

The clearest correlation between the patient's genotype and phenotype is seen in the pancreas. All patients we have observed that are homozygous for the ΔF508 deletion are PI. However, even in these patients, genetically identical at the CF locus, there is considerable variation in clinical outcome. This variation is expressed in the age of diagnosis, pulmonary function, and sweat chloride value. In the lungs of CF patients, damage is principally caused by bacterial infection. These infections are believed to be secondary to the abnormal mucus present in patients. Furthermore, immune function genes such as the human lymphocyte antigens (HLA) and/or the T cell receptor locus could play a role in the susceptibility and/or response to bacterial infection.

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