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Spectrum of Mutations in Cystic Fibrosis

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Cystic fibrosis (CF) is a disorder characterized by elevated sweat electrolytes and thick mucous secretions due to abnormal chloride permeability in epithelial tissues. The gene responsible for this disease, the CF transmembrane conductance regulator (CFTR) was identified by a positional cloning approach 3 years ago. Since that time, over two hundred mutations have been found in CFTR genes from affected individuals. Analysis of these disease-associated mutations has provided new insight into the etiology of this disease and into the mechanisms of epithelial electrolyte secretion.

KEY WORDS: CFTR gene; ethnic distribution; genotype/phenotype.

Cystic fibrosis is one of the more common, lethal genetic diseases in the Caucasian population. It affects about one in every 2500 newborns and is a predominant cause of chronic sinus, pulmonary, and pancreatic disease in children and young adults (Boat *et al.*, 1989). The locus responsible for this disorder was mapped to the long arm of chromosome 7 of man in 1985. Four years later, the defective gene was successfully identified using positional cloning techniques (Rommens *et al.*, 1989; Riordan *et al.*, 1989). The gene encompasses approximately 250,000 bases of DNA and is composed of 27 exons which are spliced to form a messenger RNA (mRNA) of 6500 base pairs. The predicted amino acid sequence of the mRNA reveals duplicated hydrophobic and ATP-binding domains surrounding a single putative regulatory domain (Riordan *et al.*, 1989) (Fig. 1). The polypeptide encoded by this gene has been named the cystic fibrosis transmembrane conductance regulator (CFTR), which appears to function as a *c*AMP-regulated chloride channel. For a review of CFTR function, see reviews by McIntosh and Cutting (1992) and Collins (1992).

To coordinate the collection and distribution of information relating to CF mutations, a consortium of almost 90 laboratories worldwide was organized by Dr. Lap-Chee Tsui. Over 200 putative mutations have been reported to the CF Genetic Consortium since its inception in 1989 (Fig. 1). Novel mutations are being reported to the Consortium at the rate of approximately 10 per month, suggesting that many more mutations in this gene are yet to be discovered. The majority of these mutations involve a single nucleotide; 42% of the reported mutations cause a change in the amino acid and are termed missense, 21% change the amino acid to a termination codon and are termed nonsense, and approximately 10% alter nucleotides known to be crucial to the proper splicing of RNA into messenger RNA. The remainder of the mutations involve an insertion or deletion of 1 or 2 nucleotides, thus changing the reading frame of the mRNA and usually introducing a premature termination signal.

One mutation has been found to occur in approximately 70% of CFTR genes from cystic fibrosis patients. The mutation is a deletion of 3 nucleotides, which causes the omission of a phenylalanine residue at position 508 in the protein. The mutation has been named deltaF508; delta being shorthand for deletion, F is the single-letter code for a phenylalanine residue, and 508 denotes its location in the protein. Although this is the most common CF mutation, its frequency varies considerably among human populations. The highest frequency has been found among Northern European populations where it accounts for 80–88% of CF mutations. This frequency drops to the 40–50%

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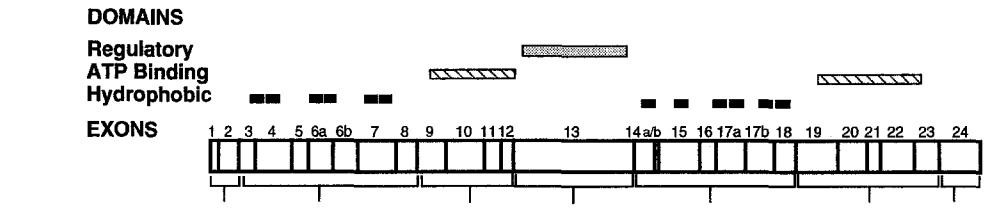

								Total
MISSENSE	2	27	22	8	21	15	0	**95**
NONSENSE	3	7	7	6	7	11	0	**41**
FRAMESHIFT	1	19	4	7	6	13	1	**51**
SPLICING	0	6	2	5	4	8	2	**27**
DELETION (in frame)	0	1	2	1	0	0	0	**4**
OTHER/UNCERTAIN	1	3	1	0	2	0	0	**7**
	7	63	38	27	40	47	3	**225**

Total (9/9/92) = 225

Fig. 1. Cystic fibrosis mutations in the CFTR gene reported by members of the CF Genetic Analysis Consortium. The relative positions of the proposed membrane-spanning hydrophobic domains, ATP-binding domains, and regulatory region are shown above the 27 exons of the gene. The relative location and nature of the 225 mutations reported to the Consortium are shown below the exons.

range in Southern European populations and was found to be even lower in the Ashkenazim and in African-Americans (Cystic Fibrosis Genetic Analysis Consortium, 1990; European Working Group on CF Genetics (EWGCFG), 1990).

The commonness of the deltaF508 mutation has prompted extensive studies of CFTR protein bearing this alteration. The mutation causes a lack of the *c*AMP-mediated chloride conduction normally attributed to CFTR. Further study reveals that CFTR with deltaF508 is improperly trafficked to the cell membrane, probably due to abnormal folding and/or post-translational modifications of the protein (Cheng *et al.*, 1990; Dalemans *et al.*, 1991; Denning *et al.*, 1992).

In addition to the deltaF508 mutation, three other deletions which leave the reading frame intact have been described. Interestingly, one of these mutations involves the isoleucine residue adjacent to the phenylalanine residue at codon 508 (Kerem, B. *et al.*, 1990). The largest alteration published to date has been 84 base pair deletion in exon 13 (Granell *et al.*, 1992). Large rearrangements involving entire or multiple exons have not been described in this gene, and neither have mutations in the promoter, in the polyadenylation signal, or in the start sites for transcription or translation.

The majority of the remaining mutations reported to the Consortium are rare, occurring on only one or two chromosomes from CF patients. However, a subset of approximately 20 mutations appear to occur in a number of Caucasian populations of European descent accounting for 2–13% of CF alleles depending on the population group (Cutting *et al.*, 1992). In ethnically discrete populations, such as Ashkenazi Jews and French Canadians in the Saguenay-Lac St. Jean region of Quebec, a few mutations account for the vast majority of CF alleles (Rozen *et al.*, 1992; Abeliovich *et al.*, 1992). It has been surmised that carriers of these mutations must have been among the founders of these ethnic groups. Marriages within these groups then maintained these mutations at higher frequencies than observed in the remainder of the Caucasian population.

In a majority of cases, the evidence that a missense mutation is disease-producing has been derived from the lack of its occurrence on chromosomes from normal individuals. Absence of a particular alteration on 100 or more normal chromosomes is generally taken as evidence that the alteration is not a neutral polymorphism. However, this approach is fraught with the problem that rare polymorphisms may be erroneously labelled as mutations. Indeed, a number of polymorphisms have been described in CFTR which are not associated with disease. Most notable is the observation that the phenylalanine residue at codon 508 can be changed to cystine and the isoleucine residues at 506 and 507 can be changed to valine without apparent effect (Kobayashi *et al.*, 1990). In certain cases, multiple missense mutations have occurred at the same residue. Examples include four changes in the serine residue at codon 549 and two changes in the glycine residue at codon 551 (Cutting *et al.*, 1990b; Kerem *et al.*, 1990; Sangiuolo *et al.*, 1991; Strong *et al.*, 1991). These observations suggest that a particular residue is functionally important for the molecule. In a similar vein, missense mutations have

been found clustered within a particular domain of the protein. This is also suggestive evidence that mutations are occurring at residues crucial for proper function of CFTR (Cutting *et al.*, 1990b). Proof of the deleterious effect of many of the rare missense mutations reported to the Consortium will require functional analysis of CFTR bearing these mutations. Thus far, heterologous expression systems have been used to examine the effect of only a few of these naturally occurring mutations (Cheng *et al.*, 1990; Drumm *et al.*, 1991).

Studies of the effect of several other types of mutation are also in preliminary stages. Three of the more common nonsense mutations cause severe reductions in mRNA levels (Hamosh *et al.*, 1991, 1992b). A similar effect has been observed with nonsense mutations in other genes. This reduction in transcript level appears to be associated with the lack of CFTR product from that allele (Zeitlin *et al.*, 1992). Patients bearing two nonsense mutations have been described with the severe form of the disease, though in some cases a very mild pulmonary lung disease has been observed (Shoshani *et al.*, 1992; Cutting *et al.*, 1990a). A number of point mutations have been reported which are proposed to affect splicing of CFTR, but only a few have been analyzed in detail (Chu *et al.*, 1991).

Although studies analyzing the association between mutation (genotype) and severity of disease (phenotype) are in early stages, some conclusions can now be drawn. The common mutation deltaF508 appears to be associated with the classic form of disease (Kerem, E. *et al.*, 1990). This includes high levels of chloride in the sweat and nonfunctioning exocrine pancreas (termed pancreatic insufficiency). However, pulmonary disease, the life-limiting feature of cystic fibrosis, appears to be variable in patients bearing this common mutation. The missense mutation, aspartic acid replacing glycine at codon 551 (G551D), is also associated with high sweat chloride levels, pancreatic insufficiency, and variable lung disease, producing a phenotype that is almost indistinguishable from patients bearing the deltaF508 mutation (Hamosh *et al.*, 1992a). These two mutations are therefore described as pancreatic insufficient or PI mutations. It has been known for a number of years that approximately 10–15% of cystic fibrosis patients have a functioning pancreas (termed pancreatic sufficiency). This feature was noted to be concordant within families, suggesting a genetic basis for this condition. Investigators in Toronto have now demonstrated that certain mutations when present in one or two CFTR genes are associated with the pancreatic sufficient or PS phenotype (Kristidis *et al.*, 1992). Finally, unusual adult patients have been described with minimal lung disease and borderline elevated strict chloride levels with and without pancreatic disease (Knowles *et al.*, 1989). These patients have been termed atypical and they appear to carry mutations that are distinct from those observed in patients with the PI or PS form of the disease (Strong *et al.*, 1991). Therefore, it appears that genotype influences some but not all aspects of the disease. For a more extensive review of this topic, see Hamosh and Cutting (1992).

In summary, newer molecular genetic techniques such as polymerase chain reaction have enabled the rapid identification of a large number of putative mutations in the gene responsible for cystic fibrosis. The distribution of alleles among human populations and possible relationships between mutation and disease severity are being investigated. The nature and location of some mutations have given clues as to the functionally important regions of CFTR. However, the importance of identifying these naturally occurring mutations will be realized upon functional analysis of CFTR bearing these mutations. This will be especially true for mutations that are more frequent in the population and mutations that are associated with unusual forms of the disease.

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