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Genetic Determination of Exocrine Pancreatic Function in Cystic Fibrosis

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Summary

We showed elsewhere that the pancreatic function status of cystic fibrosis (CF) patients could be correlated to mutations in the CF transmembrane conductance regulator (CFTR) gene. Although the majority of CF mutations—including the most common, ΔF508—strongly correlated with pancreatic insufficiency (PI), approximately 10% of the mutant alleles may confer pancreatic sufficiency (PS). To extend this observation, genomic DNA of 538 CF patients with well-documented pancreatic function status were analyzed for a series of known mutations in their CFTR genes. Only 20 of the 25 mutations tested were found in this population. They accounted for 84% of the CF chromosomes, with ΔF508 being the most frequent (71%), and the other mutations accounted for less than 5% each. A total of 30 different, complete genotypes could be determined in 394 (73%) of the patients. The data showed that each genotype was associated only with PI or only with PS, but not with both. This result is thus consistent with the hypothesis that PI and PS in CF are predisposed by the genotype at the CFTR locus; the PS phenotype occurs in patients who have one or two *mild* CFTR mutations, such as R117H, R334W, R347P, A455E, and P574H, whereas the PI phenotype occurs in patients with two *severe* alleles, such as ΔF508, ΔI507, Q493X, G542X, R553X, W1282X, 621 + 1G→T, 1717-1G→A, 556delA, 3659delC, I148T, G480C, V520F, G551D, and R560T.

Introduction

Cystic fibrosis (CF) is the most common autosomal recessive disease affecting Caucasian populations (Boat et al. 1989). It is characterized by chronic suppurative lung disease, pancreatic fibrosis that usually leads to exocrine pancreatic failure, high sweat-electrolyte concentrations, and reduced life expectancy. It has long been recognized, however, that about 15% of patients with CF have sufficient preservation of pancreatic function to prevent steatorrhea (Gibbs et al. 1950; di Sant'Agnese 1955; Corey et al. 1984; Gaskin et al. 1982*a*). As a group, the patients with pancreatic sufficiency (PS) have better pulmonary function, lower sweat-electrolyte concentrations, and better overall survival than those with pancreatic insufficiency (PI) (Gaskin et al. 1982*b;* Corey et al. 1984). A genetic difference between PI and PS patients was suggested by the concordance of pancreatic function status among affected members within the same family (Corey et al. 1989) and by strikingly different allelic and haplotype distributions of DNA markers closely linked to the CF gene locus on chromosome 7 (Kerem et al. 1989*a*). The CF gene was recently cloned and predicted to encode a protein named "cystic fibrosis transmembrane conductance regulator" (CFTR) (Kerem et al. 1989*b;* Riordan et al. 1989; Rommens et al. 1989). The most common disease-causing mutation, ΔF508, occurs in approximately 70% of CF chromosomes (Kerem et al. 1989*b*), with variable prevalence in populations of different ethnic origin (Cystic Fibrosis Genetic Analysis Consortium 1990).

We have examined, in a separate publication, the relation between genotype and phenotype in CF with

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respect to ΔF508 and have demonstrated that ΔF508 is strongly associated with both PI and earlier age of presentation (Kerem et al. 1990*a*). In addition, PS was found to occur in some patients who were compound heterozygotes for ΔF508. Over 130 additional CF alleles have now been identified, and most of them are rare (Cystic Fibrosis Genetic Analysis Consortium 1990). Preliminary analysis showed that mutations affecting the highly conserved amino acid residues in the two putative nucleotide-binding folds of the CF gene were often associated with PI (Kerem et al. 1990*b*). Two other reports suggested that patients homozygous for nonsense (stop codon) mutations could have severe pancreatic impairment but mild pulmonary disease (Cuppens et al. 1990; Cutting et al. 1990). Another report identified three point mutations—two in exon 4 and one in exon 7 of the CF gene—and all of them were associated with a mild clinical course (Dean et al. 1990). These conclusions were, however, based on small numbers of patients. Since it has been previously shown that significant variation in pulmonary function existed among homozygous ΔF508 patients (Kerem et al. 1990*a*), a good understanding of the relation between genotype and phenotype in CF would require extensive data collection. In the present report, we present both data from screening 25 different CF gene mutations in a large cohort of patients and correlation between genotype and pancreatic function status, to demonstrate that PI and PS are genetically determined at the CF gene locus.

Material and Methods

Patients

The study population consisted of 538 patients with CF who regularly attended the CF clinic at The Hospital for Sick Children in Toronto. The diagnosis of CF was made on the basis of the clinical presentation of typical pulmonary and/or gastrointestinal disease, together with at least two abnormal values on a sweat chloride test. Informed consent was obtained from the patient, parent, or legal guardian, and the study was approved by the Human Subjects Review Committee of The Hospital for Sick Children.

Each patient's clinical data, including pancreatic function status, were incorporated into a computerized data base that has existed since 1977. We classified patients as PI or PS, according to their most recent clinical status, as PI or PS, using a combination of fecal fat balance studies, serum cationic trypsinogen estimations, and pancreatic stimulation tests, as we have described in detail elsewhere (Kerem et al. 1990*a*). Each patient's pancreatic status was determined before genotype analysis was performed. Patients were also analyzed for the occurrence of neonatal meconium ileus and for a documented change in pancreatic status from PS to PI.

Genetic Analysis

Twenty-five CF gene mutations were selected for this study (table 1). With the exception of 31 siblings, 28 of whom had died, the entire study population was screened for these mutations. In most instances, siblings were assigned the same mutations even if only one affected member of the family was tested. Total human genomic DNA was isolated from the patients' peripheral blood cells, and exons 4, 7, 9–12, 19, 20, and 21 of the CFTR gene were individually amplified by PCR according to a method described elsewhere (Kerem et al. 1990*a*, 1990*b*; Zielenski et al. 1991*b*). Amplified products were immobilized onto nylon membranes and were hybridized with ^{32}P-labeled oligonucleotides specific to each of the mutations and to each of the corresponding normal DNA sequences.

Results

Of the 538 patients studied, 469 (87%) were PI, and 51 of them had died. Median age of the living PI patients was 16 years. Sixty-nine (13%) of the patients were PS, only one of whom has died. Median age of the PS patients was 26 years. Only five PS patients were below 10 years of age, and only one was below 5 years of age.

Table 2 ranks, in descending order of frequency, the number and proportion of CF alleles carrying each of the 25 mutations analyzed. ΔF508 was by far the most common (70.9%), in keeping with our previous reports (Kerem et al. 1989*b*; 1990*a*). The next most common were G551D (3.1%), G542X (2.2%), and 621 + 1G→T (1.3%). The remaining 21 mutations each occurred with a frequency of less than 1%. Twenty mutations cumulatively accounted for 84% of all CF alleles in our study group, and five mutations tested were not detected in any of the patients.

To determine the relation between genotype and pancreatic function status, we analyzed data from 394 (73.1%) of the patients for whom both CF gene mutant alleles were identified. For each CF genotype the number of patients who were PI or PS is given in table 3. The most striking observation was that, except for two cases, all given genotypes correlated with either

Table 1

Twenty-five CF Gene Mutations

Location and Mutation	DNA Change	Amino Acid Change	Reference
Exon 4:			
D110H			Dean et al. 1990
444delA	G460→C	Asp 110→His	White et al. 1991
R117H	A deletion	Frameshift	Dean et al. 1990
556delA	G482→A	Arg 117→His	Zielenski et al. 1991*a*
I148T	A deletion	Frameshift	F. Rininsland, D. Bozon, and L.-C.
	T575→C	Ile 148→Thr	Tsui, unpublished data
Intron 4:			
621 + 1G→T ...	621 + 1G→T	Splice mutation	Zielenski et al. 1991*a*
Exon 7:			
R334W	C1132→T	Arg 334→Trp	Gasparini et al. 1991
R347P	C1172→G	Arg 347→Pro	Dean et al. 1990
Exon 9:			
A455E	C1496→A	Ala 455→Glu	Kerem et al. 1990*b*
G458V	G1505→T	Gly 458→Val	Cuppens et al. 1990
G480C	G1570→T	Gly480→Cys	Strong et al. 1991
Exon 10:			
Q493X	C1609→T	Gln 493→stop	Kerem et al. 1990*b*
ΔI507	3-bp deletion	del of Ile 507	Kerem et al. 1990*b*
ΔF508	3-bp deletion	del of Phe 508	Kerem et al. 1989*b*
V520F	G1690→T	Val 520→Phe	Jones et al. 1991
Intron 10:			
1717-1G→A ...	G1717-1→A	Splice mutation	Kerem et al. 1990*b*
Exon 11:			
G542X	G1756→T	Gly 542→stop	Kerem et al. 1990*b*
S549R	T1779→G	Ser 549→Arg	Kerem et al. 1990*b*
G551D	G1784→A	Gly 551→Asp	Cutting et al. 1990
R553X	C1789→T	Arg 553→stop	Cutting et al. 1990
R560T	G1811→C	Arg 560→Thr	Kerem et al. 1990*b*
Exon 12:			
Y563N	T1819→A	Tyr 563→Asn	Kerem et al. 1990*b*
P574H	C1853→A	Pro 574→His	Kerem et al. 1990*b*
Exon 19:			
3659delC	C deletion	Frameshift	Kerem et al. 1990*b*
Exon 20:			
W1282X	G3978→A	Trp 1282→stop	Vidaud et al. 1990
Exon 21:			
N1303K	C4041→G	Asn 1303→Lys	Osborne et al. 1990

PI or PS, but not with both. The exceptions were two patients, among the 279 patients homozygous for ΔF508, who were PS instead of PI. At the conclusion of this study, however, it was noted that both of these patients had since produced abnormal fecal fat, indicative of steatorrhea (data not shown). In fact, the result of a previous co-lipase secretion test for one of the patients was close to the threshold for steatorrhea, already signifying severe pancreatic dysfunction (Gaskin et al. 1982*a*). Retrospective clinical analysis revealed that 15 of the current PI patients (11 of whom were homozygous for ΔF508) had been PS earlier in life.

In a previous report, we hypothesized that patients with two *severe* CF mutations would be expected to be PI, and that patients with two *mild* mutations or one *mild* plus one *severe* mutation would be expected to be PS (Kerem et al. 1989*b*). The result shown in table 3 was therefore in complete agreement with this prediction.

Patients who had neonatal meconium ileus were also identified. As shown in table 3, meconium ileus

Table 2

Frequency of 25 CF Mutations in Chromosomes of the Toronto Study Population

Mutation	Frequency (no. of chromosomes detected)	% of Total CF Alleles	Cumulative Fraction (%)
ΔF508	632	70.9	70.9
G551D	28	3.1	74.0
G542X	20	2.2	76.2
621+1G→T ...	12	1.3	77.5
N1303K	8	.9	78.4
W1282X	8	.9	79.3
R117H	8	.9	80.2
1717-1G→A ...	5	.6	80.8
R560T	5	.6	81.4
ΔI507	5	.6	82.0
R553X	4	.4	82.4
V520F	3	.3	82.7
R334W	3	.3	83.0
A455E	2	.2	83.2
I148T	2	.2	83.4
Q493X	2	.2	83.6
P574H	1	.1	83.7
R347P	1	.1	83.8
556delA	1	.1	83.9
3659delC	1	.1	84.0
G480C	1	.1	84.1
444delA	0	0	84.1
D110H	0	0	84.1
G458V	0	0	84.1
S549R	0	0	84.1
Y563N	0	0	84.1
Overall	752	84.1	84.1

Table 3

Complete CF Genotypes for 394 Patients

Genotype		No. of Patients with	
Allele 1	Allele 2	PI[a]	PS
ΔF508	ΔF508	277 (49)	2[b]
	G551D	21 (1)	0
	G542X	18 (9)[c]	0
	621+1G→T	11 (1)	0
	ΔI507	7 (1)	0
	N1303K	6 (1)	0
	R560T	5	0
	1717-1G→A	5 (1)	0
	556delA	3	0
	Q493X	3	0
	R553X	3 (1)	0
	W1282X	3	0
	3659delC	2	0
	I148T	1	0
	R117H	0	9
	A445E	0	2
	P574H	0	2
	R347P	0	1
G551D	1717-1G→A	2	0
	621+1G→T	1	0
	G480C	1	0
	G551D	1	0
	V520F	1 (1)	0
G542X	V520F	1	0
I148T	W1282X	1 (1)	0
W1282X	W1282X	1	0
N1303K	R553X	1 (1)	0
R117H	R117H	0	1
	G542X	0	1
R334W	R334W	0	1

[a] Numbers in parentheses are number of patients with neonatal meconium ileus.

[b] For explanation, see text.

[c] Unusually high.

occurred in 80 (17%) of the PI patients and in none of the PS patients. Although meconium ileus was found in patients who had diverse genotypes, it appeared to be overrepresented (9/18) in patients who had the ΔF508/G542X genotype.

The remaining 121 patients each had one known and one unknown CF mutant allele. In accordance with our hypothesis discussed below, only one of these patients had a known *mild* mutation (R117H) and was PS. In 23 patients, none of the 25 tested mutations were found.

Discussion

In light of the large number of patients analyzed, this study strengthens the hypothesis that pancreatic-function status in CF is genetically determined by specific mutations at the CF gene locus (Kerem et al. 1989*b*, 1990*a*, 1990*b*; Zielenski et al. 1991*a*). Our data strongly support the hypothesis that, with respect to pancreatic function, *mild* mutant alleles confer a higher residual CFTR activity than do *severe* mutant alleles. Although PS occurs in patients who have one or two *mild* mutations, PI occurs in patients who are homozygous for or who are genetic compounds of two *severe* mutant alleles.

On the basis of both the preceding hypothesis and our present data, it was then possible to classify mutations as *severe* or *mild* with respect to pancreatic function (table 4). For example, the *severe* ΔF508 mutation combined with any of the other *severe* mutations was associated with PI, whereas ΔF508 combined

Table 4

Classification of CF Gene Mutations as Severe or Mild with Respect to Pancreatic Function

Type of Mutation	Severe (location)	Mild (location)
Missense (point mutation)	I148T (exon 4)	R117H (exon 4)
	G480C (exon 9)	R334W (exon 7)
	V520F (exon 10)	
	G551D (exon 11)	R347P (exon 7)
	R560T (exon 11)	A455E (exon 9)
	N1303K (exon 21)	P574H (exon 12)
Single amino acid deletion	ΔF508 (exon 10)	
	ΔI507 (exon 10)	
Stop codon (nonsense)	Q493X (exon 10)	
	G542X (exon 11)	
	R553X (exon 11)	
	W1282X (exon 20)	
Splice junction ...	621 + 1G→T (intron 4)	
	1717-1G→T (intron 10)	
Frameshift	556delA (exon 4)	
	3659delC (exon 19)	

with any of the *mild* mutations was associated with PS. From table 4 it can be seen that all the stop codon, splice junction, and frameshift mutations were *severe*. Two single amino acid deletions in exon 10—ΔF508 and ΔI507—were also *severe*. Missense mutations, caused by single nucleotide changes, could be *severe* or *mild*. Accordingly, the mutations R117H, R334W, R347P, A455E, and P574H may be regarded as *mild*, whereas ΔF508, ΔI507, Q493X, G542X, R553X, W1282X, 621 + 1G→T, 1717-1G→A, 556delA, 3659delC, I148T, G480C, V520F, G551D, and R560T are *severe*.

In our study cohort of 538 CF patients, only two of the 538 patients, who were homozygous for ΔF508 and PS, were exceptions to the above classification. Subsequent follow-up studies revealed that these two patients had developed signs of steatorrhea. In fact, in longitudinal studies, deterioration of pancreatic function with age had been documented (Durie et al. 1988). Waters et al. (1990) also reported that 37% of CF infants diagnosed by newborn screening were PS but that after a mean follow-up after 2–3 years, only 29% remained PS. The reason why this transition occurred in only a subset of patients is not understood. In our population, for which newborn screening is not performed, only 13% (69 patients) are PS, and of these, only one is less than 5 years of age. The overall consistency of our data and the older age of our PS patients suggest that change in pancreatic function status most commonly occurs in early childhood and that most of our current PS patients will remain PS.

In general, PI patients have more severe pulmonary disease and a poorer long-term prognosis than do PS patients (Gaskin et al. 1982*b*; Corey et al. 1984). There is, however, wide variability of pulmonary function even among patients with the most common PI genotype (ΔF508/ΔF508) (Kerem et al. 1990*a*). Extremely mild presentation of pulmonary disease has been reported in several adult homozygous ΔF508 patients (Santis et al. 1990). In view of these observations, correlations of specific genotypes and pulmonary function may not be straightforward. Overall trends may only be apparent in a large group of patients, which, for many rare genotypes, will only be possible to determine if multi-center studies are carried out. For example, nine of 18 patients with the ΔF508/G542X genotype have been found to have neonatal meconium ileus; this frequency is much above the overall proportion observed in our clinic (17%). The significance of this observation is unclear, and additional studies with larger sample sizes are required, especially since other patients with nonsense mutations (including one homozygous for W1282X) were not found to be associated with meconium ileus.

Delineation of *severe* and *mild* CF gene mutations should aid in further studies of the structure and function of the CFTR protein (Riordan et al. 1989). Earlier studies indicated that secretion of water, chloride, and bicarbonate is impaired in the CF pancreas (Kopelman et al. 1988), leading to protein hyperconcentration and ductular obstruction (Kopelman et al. 1985). In vitro studies suggested that a relative impermeability of apical cell membranes to chloride is the molecular defect in CF (Li et al. 1985, 1989). Furthermore, DNA transfection studies show that CFTR is a cAMP-regulated chloride channel (Anderson et al. 1991*b*; Kartner et al. 1991; Rommens et al. 1991) In addition, some *severe* CF mutations including ΔF508 appear to result in incomplete processing of CFTR—and hence its absence from its correct cellular location (Cheng et al. 1990). It is therefore of interest to note that all of the *mild* CF mutations studied here involve missense mutations. The mild nature of these mutations may be due to either a reduced amount of CFTR in the correct cellular location, or a normal quantity of protein that functions suboptimally. In support of our observation, the results of a more recent DNA transfection study show that certain amino acid substitutions in the predicted transmembrane domain of

CFTR could lead to an alteration of anion selectivity (Anderson et al. 1991*a*).

Although the decision whether to do population screening for any genetic disease is ultimately a sociological one, it has been argued on the basis of risk calculation that CF carrier screening should only be offered if the sensitivity of detection reaches at least 90% (Beaudet et al. 1990; Gilbert et al. 1990). After testing for 25 relatively frequent mutations in 538 CF patients in our clinic, however, the cumulative proportion of CF alleles covered is only 84%. While our mutation analysis continues, all available evidence, including results from direct sequencing of patient DNA and extensive haplotype examination, indicates that, since the majority of CF alleles have already been identified, the remaining CF alleles are probably rare. Therefore, it is unlikely that the sensitivity of carrier testing in the general population in Canada as well as in most other geographic locations in the world (with only a few exceptions) would reach the required 90%. To avoid fostering unnecessary anxiety and stigmatization in couples with a single identified carrier, we concur with the recommendation that services such as public education and genetic counseling service should be established before any large-scale carrier screening is implemented.

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