Thirteen cystic fibrosis patients, 12 compound heterozygous and one homozygous for the missense mutation G85E: a pancreatic sufficiency/insufficiency mutation with variable clinical presentation

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Abstract

To study the severity of mutation G85E, located in the first membrane spanning domain of the CFTR gene, we studied the clinical features of 13 Spanish patients with cystic fibrosis (CF) carrying this mutation. G85E accounts for about 1% of Spanish CF alleles. One patient was homozygous G85E/G85E and the rest were compound heterozygotes for G85E and other mutations ($\Delta F508$ nine patients, $\Delta I507$ two patients, and $712-1G>T$ one patient). The characteristics of the pooled G85E/any mutation group were compared with those of 30 $\Delta F508$ homozygotes. Mean age at diagnosis and percentage of ideal height for age were higher in the G85E/any mutation group (4.2 (SD 4.7) v 2.4 (SD 2.3), $p<0.05$, and 102.8 (SD 4.7) v 97.8 (SD 4.1), $p<0.01$), both probably related to the greater prevalence of pancreatic sufficiency (70% v 0%, $p<0.01$). The G85E homozygote was pancreatic sufficient. Sweat sodium levels were slightly higher, and salt loss related problems more frequent, in the G85E/any group. Two of the G85E patients died of respiratory failure aged 6 and 14 years. Striking discordance in the phenotype was observed in two pairs of sibs, one of them dizygotic twins, suggesting that factors, genetic and environmental, other than CFTR genotype are important in determining CF phenotype. (*J Med Genet* 1996;33:820-822)

Key words: cystic fibrosis; CFTR mutations; phenotype; G85E mutation.

Cystic fibrosis (CF) is the commonest life threatening autosomal recessive disorder in white populations, with an incidence of 1/2000 to 1/4000.¹ The gene responsible for the disease encodes the CF transmembrane conductance regulator protein (CFTR).^{2,3} Over 500 different mutations have been identified. The frequency of most of these is very low, and varies considerably among different populations.⁴

Clinical manifestations of CF include pancreatic insufficiency (PI), chronic pulmonary disease, raised sweat chloride and sodium

levels, and bilateral absence of the vas deferens. A number of genotype-phenotype correlations have been studied and have shown that while the link between genotype and pancreatic function is usually strong, correlations with other characteristics are far more elusive.⁵ Some of the features of classical CF may sometimes be lacking leading to "mild" or "atypical" forms.⁶⁻¹⁷ Many mutations associated with such phenotypes are missense substitutions in the membrane spanning domains (MSD) of the CFTR.^{7-9,15} Some of these mutant proteins are normally processed and located in the apical membrane, and lead to cAMP regulated Cl-currents,¹⁵ although smaller than normal (class IV mutations⁶). Conversely missense mutations located in the nucleotide binding folds are usually severe.¹⁸ Other mutations associated with mild CF are some splicing mutations in which low levels of normal transcripts are produced.^{16,17} We present here the clinical findings in 13 Spanish CF patients carrying the missense mutation G85E, a G to A change at nucleotide position 386 in exon 3, which results in a glutamic acid for glycine substitution at codon 85 in the first MSD of the CFTR.¹⁹

Clinical data of the patients were obtained from several centres. Patients 7-12 were under care at Hospital de Cruces and patients 1-6 were each at different hospitals around the country. Another hospital (Aranzazu, San Sebastian) provided the clinical data of patient 13, an older sib of patient 8, who died before CF genotyping was feasible. The clinical features of patients with a G85E/any mutation genotype were compared with those of 30 $\Delta F508$ homozygotes under control at Hospital de Cruces. For the statistical analysis, in the case of continuous variables, means were compared by unpaired two tailed Student's *t* and Mann-Whitney tests. Chi squared analysis was used to assess differences between groups for categorical variables. A *p* value <0.05 was considered significant.

Pancreatic status was assessed by quantitative fat excretion analysis and determination of stool chymotrypsin concentrations.²⁰ These data were not available for four patients in whom we relied on their clinical need for pancreatic enzymes. The best FEV1 over the last year was chosen as an index of pulmonary

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Table 1 Clinical features of 13 CF patients carrying the G85E mutation compared with $\Delta F508$ homozygotes

Parameter	Genotype	
	G85E/any	$\Delta F508/\Delta F508$
No of patients	13	30
Sex: female/male	6/7	12/18
Alive	11	28
	Mean, SD (No studied)	
Current age (y)	8.4, 5.4 (11)	9.9, 4.3 (28)
Age at diagnosis (y)	4.23, 4.7 (13)	2.4, 2.3 (30)*
Sweat sodium (mmol/l)	105.1, 17.0 (7)	94.3, 14.0 (23)*
Sweat chloride (mmol/l)	107.2, 4.0 (5)	97.5, 14.0 (22)
Schwachman score	87.5, 7.5 (10)	88.7, 9.4 (28)
Chrispin-Norman chest x ray score	4.5, 3.1 (9)	4.7, 5.3 (27)
FEV1 (% predicted)	80.2, 8.2 (6)	87.1, 13 (24)
FVC (% predicted)	85.3, 7.7 (6)	94.8, 14.6 (24)
% Ideal body weight	101.4, 14.2 (10)	100.7, 11.2 (28)
% Ideal body height	102.8, 4.7 (10)	97.8, 4.1 (28)**
	No positive/No studied (%)	
Pancreatic insufficiency	4/13 (30.7)	30/30 (100)**
Colonisation with <i>P aeruginosa</i>	10/13 (76.9)	24/30 (80)
Chronic bronchial infection with <i>P aeruginosa</i>	5/13 (38.4)	10/30 (33.3)
Meconium ileus	0/13 (0)	2/30 (6.7)
Liver disease	1/13 (7.6)	2/30 (6.7)
Dehydration and metabolic alkalosis	4/13 (30.7)	1/30 (3.4)*

*p<0.05, **p<0.01.

function. Age at which a first sputum or throat swab positive for *Pseudomonas aeruginosa* was obtained ("colonisation"), and presence of chronic bronchial infection (CBI) with *P aeruginosa*, were recorded. Ideal weight for height and height for age were determined from growth charts for Spanish children. Only sweat samples collected with either the preweighted gauze Gibson and Cooke method or the Wescor Macroduct system²¹ were used for comparison.

Screening by DGGE²² of 50 DNA samples with unknown mutations showed one sample with an abnormal pattern in exon 3. Direct sequencing allowed us to identify the G85E mutation. The microsatellite haplotype 16-24-13 (IVS8CA-IVS17bTA-IVS17bCA)²³ was used to study CF samples with uncharacterised mutations from 160 CF patients. G85E was found in 13 samples.

All chromosomes with mutation G85E were associated with the same microsatellite haplotype 16-24-13. Nine patients were compound heterozygotes for $\Delta F508$ /G85E, one pair of sibs were G85E/ $\Delta I507$, one was G85E/712-1 G>T, and one was a G85E homozygote. Data from G85E/ $\Delta F508$ and G85E/non- $\Delta F508$ patients were pooled for comparison with those from $\Delta F508$ homozygotes.

The G85E/any mutation patients were older at diagnosis, had a higher percentage of ideal height, and a lower prevalence of PI (30% v 100%, p<0.01) compared with the $\Delta F508$ homozygotes (table 1). Sweat sodium was higher and there was a greater prevalence of dehydration-hypochloreaemic metabolic alkalosis in the former. No other difference in the variables studied was found. Two patients in each group died of severe respiratory disease. Ages at death were 6 and 14 years in the G85E/any group, and 16 and 18 years in the $\Delta F508$ homozygous group.

Table 2 shows the clinical features of the 13 patients, exhibiting a wide variability in clinical presentation. The G85E homozygote was a 17 year old girl with pancreatic sufficiency (PS), who had recurrent coughing from the age of 4 and was diagnosed at the age of 13 years. She had mild pulmonary function impairment without CBI. Two pairs of sibs (patients 8 and 13 and 11 and 12) were notable for their discordant phenotype. Patient 13 had classical CF with PI and severe respiratory disease and died of respiratory failure aged 6. His younger sib (patient 8) had PS and remained largely free of respiratory symptoms at the age of 8, with forced expiratory flows and chest x ray within normal limits. Patients 11 and 12 were dizygotic twins. While the boy had mild pulmonary disease with normal spirometry, had PI, and showed clinical and ultrasound evidence of portal hypertension, his twin sister had PS, was free of liver disease, and had moderate pulmonary disease.

The worldwide prevalence of G85E is 0.2%,⁴ but higher frequencies have been reported in the US (0.7%),²⁴ Italy (1.7%),²⁵ and Spain (1%) (T Casals, personal communication). This mutation causes the substitution of a negatively charged amino acid for an uncharged one within the first MSD. The molecular mechanism of the CFTR dysfunction associated with G85E has recently been found to consist of a trafficking defect (class II mutation)⁶ with rapid degradation of the mutant protein before transit through the Golgi complex.²⁶ There have been only three reports on the clinical characteristics associated with this mutation. Chalkley and Harris²⁷ described an 11 year old boy, homozygous for G85E, with PS. Kerem *et al*²⁸ reported five G85E homozygotes and eight compound

Table 2 Characteristics of 13 CF patients with mutation G85E*

Patient No	Sex	Age/age at diagnosis (y)	Pancreatic status	Schwachman	CBI	FEV1 (% pred)	Other clinical features
1	F	17.6/13.3	PS	95	No	75	Mild pulmonary disease
2	F	14 (dead)/0.7	PS†	—	Yes	28	Dehydration. Severe pulmonary disease
3	M	5/0.2	PI†	75	Yes	—	Dehydration
4	M	6.7/2.2	PI†	90	No	—	Nasal polyps. Mild pulmonary disease
5	F	10.4/10	PS	80	Yes	94	Mild pulmonary disease
6	M	0.9/0.2	PS†	—	No	—	—
7	M	7.5/0.3	PS	85	Yes	72	—
8	M	8/4.8	PS	95	No	83	Minimal symptoms. Diagnosis because of family history
9	F	5/0.3	PS	95	No	—	Dehydration. Mild pulmonary disease
10	F	5.2/0.3	PS	95	No	—	Dehydration. Allergic bronchopulmonary aspergillosis
11	M	14.7/9.7	PI	90	No	86	Liver disease. Mild pulmonary disease
12	F	14.7/9.7	PS	80	No	60	Moderate pulmonary disease
13	M	6.5 (dead)/4	PI	—	Yes	—	Severe pulmonary disease. Older sib of patient 8

* Patient 1 was G85E/G85E, patients 8 and 13 G85E/ $\Delta I507$, patient 9 G85E/712-1G>T, and the rest G85E/ $\Delta F508$.

† Assessed clinically only.

heterozygotes, only two (15.3%) with PS. Friedman *et al*²⁴ reported that six out of their nine patients with G85E had PS.

The data presented here suggest that G85E is predominantly associated with PS. This inconsistent association of G85E with PS, and the clinical discordance in our two pairs of sibs, suggest that genetic factors other than CF genotype, as well as environmental factors, are involved in the severity of the disease. These factors seem more evident when the CF mutations are mild, as has been shown for mutation R334W⁷ or for the IVS8-6(5T) mutation, which is associated with male infertility, mild CF, or even a normal phenotype.⁸

In summary G85E occurred with PS in 70% of the cases. Compared with $\Delta F508$ homozygotes, patients with G85E were diagnosed later, were taller, and had a higher prevalence of dehydration, but no other difference was observed either in the severity of the pulmonary disease or in the prevalence of other CF related complications. This mutation also shows intrafamilial variability in its clinical presentation. Further experience over a longer time span is needed to clarify the comparative severity of the phenotype associated with G85E.

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