Clinical features of cystic fibrosis patients with rare genotypes

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Abstract

We describe the clinical features of seven cystic fibrosis patients from southern Italy who bear rare genotypes: (1) a patient homozygous for the 2183 AA→G mutation who was affected by a very early pulmonary form of cystic fibrosis, and five patients who were compound heterozygotes either for the 2183 AA→G mutation or for the I148T mutation, in both instances with the ΔF508 mutation; and (2) a patient homozygous for the early nonsense R553X mutation who showed only a moderately severe form of cystic fibrosis. Our results confirm that environmental or genetic factors unrelated to the CF disease contribute significantly to the development of the phenotype.

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Key words: cystic fibrosis; R553X mutation; 2183 AA→G mutation; I148T mutation.

In cystic fibrosis (CF) patients from southern Italy the ΔF508 microdeletion has a frequency of about 50%¹ versus 70 to 80% reported for CF patients from northern Europe.² Therefore, while the molecular diagnosis of CF in our area is more difficult and cumbersome, our population represents a rich source of cases bearing unusual CF genotypes. The study of the correlation between these rare genotypes, particularly in the homozygous state, and the clinical expression of the disease will greatly contribute to the elucidation of the genotype-phenotype correlation in CF patients.

We are now analysing all the known CF patients from the Campania and Lucania regions (southern Italy) using a semi-automated allele specific oligonucleotide (ASO) dot blot procedure.³ With this method we identified a panel of the most frequent CF mutations in our geographical area.⁴ During this screening, several CF patients with rare genotypes came to light. Here we report the clinical findings of CF compound heterozygotes for the I148T/ΔF508 mutations and heterozygotes or homozygotes for the 2183 AA→G mutation.⁵⁻⁷ In addition, we describe the phenotype of a CF patient homozygous for the R553X mutation which, to our knowledge, is the third such case published.

Methods

The CF mutations were analysed with a semi-automated allele specific oligonucleotide (ASO) dot blot procedure using the Biomek 1000 workstation (Beckman), which is based

on two previously described multiplex PCR amplifications of the CF gene³ at 11 DNA segments (eight exon and three intron segments). Subsequent studies, in which the analytical panel included the 2183 AA→G mutation detected by ASO, showed the method was highly reproducible.⁴ The extragenic XV2c and KM19 polymorphisms were analysed with the *TaqI* and *PstI* restriction enzymes.^{8,9} For both the analyses, allele 1 corresponds to the absence of the restriction site and allele 2 to its presence. The intron 8 VNDR was analysed by a polymerase chain reaction (PCR) amplification followed by polyacrylamide electrophoresis.¹⁰ The haplotype numbering corresponds to the number of repeats.

For each patient, the present age, the age at diagnosis, the sweat test for chloride and sodium, meconium ileus, diabetes, and nasal polyposis were recorded. The pulmonary evaluation was based on the physical examination of the patients and on the chest x ray evaluation of the Chrispin-Norman score. In addition, for patients over 6 years old the forced vital capacity (FVC) and the forced expiratory volume in one second (FEV1) were measured and expressed as a percentage of the predicted normal value for sex and height. Finally, the age at lung colonisation by *Pseudomonas aeruginosa* was also recorded. The pancreatic evaluation was based on physical examination of the patients, fat balance, and steatocrit. Finally, liver involvement was evaluated on the basis of the physical examination, ultrasound scanning, and clinical biochemistry (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ-glutamyltransferase, albumin, and Quick).

Case reports

The clinical features of the seven patients described below are listed in the table.

I148T COMPOUND HETEROZYGOTES

Two unrelated CF patients, compound heterozygotes for the I148T/ΔF508 mutations (figure, panel A), were analysed. One patient, a female, was diagnosed at the age of 3 months on account of failure to thrive, recurrent bronchiolitis, and chronic diarrhoea. She was pancreatic insufficient and her sputum was colonised by *Pseudomonas aeruginosa* since she was 7 years old. The course of the disease was characterised by mild intestinal problems, normal growth, absence of diabetes and hepatopathy, and severe pulmonary involvement (FEV1 = 37% of predicted at 10 years), with

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Clinical features of the seven cystic fibrosis patients described

	1	2	3	4	5	6	7
Sex	M	M	F	F	F	F	M
Present age	8	4	died (16 y)	4	2	4	21
Age at diagnosis	3 mth	6 mth	3 mth	1 mth	6 mth	1 y	2 y
Sweat chloride* (mEq/l)	93	93	98	96	101	117	90
Sweat sodium* (mEq/l)	67	70	75	64	76	103	115
Meconium ileus	No	No	No	No	No	No	No
Diabetes	No	No	No	No	No	No	No
Nasal polyposis	No	No	No	No	No	No	Yes
Liver involvement	No	No	No	No	No	No	No
Age of colonisation by <i>P. aeruginosa</i>	No	No	7 y	2 mth	6 mth	No	7 y
Respiratory insufficiency	No	No	Yes	No	No	No	No
Chrispin-Norman score	9	7.5	23	7	5	7	8
Pancreatic insufficiency	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Genotype	R553X R553X	Δ F508 I148T	Δ F508 I148T	2183 AA→G 2183 AA→G	Δ F508 2183 AA→G	Δ F508 2183 AA→G	Δ F508 2183 AA→G

* Cut-off = 60 mEq/l for both sweat chloride and sodium.

massive haemoptysis since the age of 15 years; the patient died at the age of 16 years.

The other case is a 4 year old boy, diagnosed at the age of 6 months on the basis of chronic diarrhoea, recurrent purulent nasal discharge, and one episode of dehydration with hypochloraemic alkalosis during the summer. He is pancreatic insufficient, steatorrhoea is well controlled with pancreatic extracts, and he has a very good nutritional status. Neither significant

pulmonary complications nor sputum colonisation has occurred. The XV2c and KM19 haplotypes were 1,1 and 2,2, respectively, and the intron 8 VNDR pattern was 23,23 for both cases. In our series of 246 CF chromosomes from our region, the frequency of the I148T mutation is 0.8% (unpublished results).

2183 AA→G HOMOZYGOTES AND HETEROZYGOTES

We studied four unrelated CF patients bearing the 2183 AA→G mutation, one homozygote and three compound heterozygotes with Δ F508 (figure, panel B). The homozygous patient is a 4 year old girl diagnosed at the age of 1 month on account of failure to thrive, steatorrhoea, and respiratory distress with atelectasia of the right upper pulmonary lobe and *P. aeruginosa* in pharyngeal aspirate cultures. She improved with pancreatic enzyme supplementation and, at present, has a good nutritional status and moderate pulmonary symptoms; her sputum is colonised by *P. aeruginosa* in spite of early, intensive, antibiotic therapy.

The heterozygous patients are one male of 21 years and two females of 4 and 2 years. They were diagnosed at the ages of 2 years, 1 year, and 6 months, respectively, on account of failure to thrive with chronic diarrhoea, steatorrhoea, and recurrent respiratory symptoms. The 21 year old male patient, who is pancreatic insufficient, has sputum colonised by *P. aeruginosa* (since the age of 7 years) and was surgically treated three times for nasal polyposis. He has never had diabetic or hepatic complications and was affected by mild pneumonopathy until the age of 17 years (FEV1 82% of predicted); pulmonary involvement has increased in the last three years causing a decline in pulmonary function and the FEV1 is now 49% of predicted.

The two girls show pancreatic insufficiency. Lung involvement was early with atelectasia of the right medium lobe in one patient and early presence of *P. aeruginosa* in sputum culture in the other. Both these problems were successfully treated with antibiotics and chest physiotherapy and, at present, the two patients have only mild respiratory symptoms.

In all four cases, the 2183 AA→G mutation is associated with alleles 1 and 2 (XV2c and

Allele specific oligonucleotide dot blot analysis of CF mutations: Panel A: 1=positive homozygous control for the Δ F508 mutation; 2 and 3=compound heterozygotes for I148T/ Δ F508; 4=healthy subject. Panel B: 1=positive heterozygous control for the 2183 AA→G mutation; 2=positive heterozygous control for the Δ F508 mutation; 3=homozygote for 2183 AA→G mutation; 4 to 6=three compound heterozygotes for 2183 AA→G/ Δ F508; 7=healthy subject. Panel C: 1=positive heterozygous control for R553X; 2=homozygous R553X CF patient; 3 and 4=father and mother of 2, both heterozygous for R553X; 5=healthy subject.

KM19, respectively) and with allele 23 (intron 8 VNDR). The incidence of the 2183 AA→G mutation in our sample of 246 CF chromosomes is 2.0% (unpublished results).

R553X HOMOZYGOTE

This male was diagnosed as suffering from CF at the age of 3 months on the basis of failure to thrive, diarrhoea, and sweat test. He is the only child of healthy parents (third degree relatives) from Campania (southern Italy). At present, at the age of 8 years, he has mild pulmonary involvement (FEV1 86% and FVC 76%), with sporadic presence of *P aeruginosa* in the sputum. Neither meconium ileus nor diabetes was present. From the steatocrit and clinical course, the patient was defined as pancreatic insufficient with a poor response to pancreatic enzyme supplementation indicated by failure to thrive and steatorrhoea. The physical examination (mild hepatomegaly), clinical biochemistry (AST, ALT, AP, and GGT increased less than twice the upper limit), and ultrasound scanning (dyshomogeneity of the parenchyma) indicated mild hepatic involvement.

The R553X mutation (figure, panel C) is associated with alleles 1,1 for the XV2c and KM19 polymorphisms and with allele 16 (intron 8 VNDR). The incidence of the R553X mutation in our population of 246 CF chromosomes is 1.2% (unpublished results).

Discussion

The cloning of the cystic fibrosis transmembrane regulator (CFTR) gene and the characterisation of the molecular defects have resulted in numerous studies on genotype-phenotype correlations, and on the relationship between specific mutations and the clinical heterogeneity of the disease.¹¹⁻¹³

The I148T mutation has been identified in 0.2% of Canadian patients⁶ and in 0.08% of French patients⁷; the latter study gives an incidence of 0.9% for the 2183 AA→G mutation and in a recent paper on CF patients from northern Italy the incidence of the 2183 AA→G mutation was 9.33%.¹⁴ These figures compare with an incidence of 0.8% for I148T and of 2.0% for 2183 AA→G in our region.

All I148T and 2183 AA→G CF cases described here presented with pancreatic insufficiency. All our patients also showed various degrees of pulmonary involvement, but no conclusion can be drawn as to the severity of lung disease and the presence of $\Delta F508$, I148T, and 2183 AA→G genotypes, because most of our patients are still very young and no comparable published data are available. Thus, we are unable to contribute to the debate¹¹⁻¹³ about the correlation between genotype and lung involvement. The early expression of pneumonopathy in the patient homozygous for 2183 AA→G and in two of the three compound heterozygous cases for the same mutation, together with the early colonisation of the sputum by *P aeruginosa*, which is very unusual in pre-school children, suggests a poor prognosis for

lung involvement in these patients. Our results agree with those obtained in three CF patients homozygous for the 2183 AA→G mutation.¹⁴ All the cases with I148T and 2183 AA→G were diagnosed within the first year of life, which is indicative of the severity of these mutations. This severity coincides with the "frameshift" nature of the mutations.

The two previously reported cases homozygous for R553X are heterogeneous, one being characterised by a moderate-severe form of the disease with lung and pancreas involvement,¹⁵ the other by very mild pulmonary involvement.¹⁶ Our patient showed mild pulmonary involvement and pancreatic insufficiency and hence shows an intermediate phenotype. The rare haplotype associated with this homozygous R553X patient (1,1 for both the XV2c and KM19 polymorphisms, and 16, 16 for the intron 8 VNDR) was also detected in one of the two homozygous R553X cases described,¹⁶ the other case being heterozygous for both the XV2c and KM19 markers.¹⁵ These observations support the hypothesis that the R553X mutation occurred more than once during evolution, as has also been described for the R117H mutation.¹⁷

Thus, the three homozygous R553X cases appear to be associated with a moderate form of cystic fibrosis, with a phenotype less severe than that usually found in $\Delta F508$ homozygous patients, which is surprising because R553X is a nonsense mutation. As previously suggested,¹⁶ the complete absence of CFTR production could be better tolerated by cells and tissues than the production of a mutated protein, even if the R553X mutation results in a very low percentage of wild type transcripts.^{18,19}

In conclusion, the three cases bearing the 2183 AA→G mutation exhibit early pulmonary expression of the disease, and this feature is more evident in the 2183 AA→G homozygous patient. The R553X mutation in the homozygous form seems to express a moderate CF phenotype analogous to one of the two previously described cases homozygous for the R553X mutation. Finally, the two patients bearing the I148T mutation were heterogeneous for both clinical features and age; however, given the lack of previous reports of this type, other case reports are required in order to establish a genotype/phenotype correlation.

The results reported in this study also support the view that environmental factors, in addition to genetic factors, are related to the clinical heterogeneity of CF patients.

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