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The CFTR 3849+10kbC->T and 2789+5G->A alleles are associated with a mild CF phenotype

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ABSTRACT: Most cystic fibrosis (CF) transmembrane receptor mutations are rare. The French CF Registry offers an opportunity to study the genotype–phenotype relationship of these rare alleles.

Since 1992, 39 CF patients carrying one copy of the 3849+10kbC->T mutation and 88 the 2789+5G->A allele have been seen at least once in a CF care centre. Among them, 16 carrying the 3849+10kbC->T/ΔF508 genotype and 34 with the 2789+5G->A/ΔF508 genotype were seen in 2000. Their age at diagnosis, sweat chloride concentration, anthropometric and lung function results, and clinical aspects were compared with those homozygous for the ΔF508 mutation matched for sex, age and CF care centre.

Major differences, most of them statistically significant, in the age at diagnosis, prevalence of pancreatic insufficiency, and other clinical signs, anthropometric and lung function measures were observed between both compound heterozygote groups and their matched ΔF508/ΔF508 groups. The mean sweat chloride concentration was also lower (close to normal values) among 3849+10kbC->T/ΔF508 patients, but not among 2789+5G->A/ΔF508 patients.

In conclusion, both mutations studied here are associated with a milder course of cystic fibrosis disease. The 3849+10kbC->T and 2789+5G->A alleles are splice site mutations, leading to abnormal mRNA; however, a small amount of normally spliced transcripts can also be detected. The presence of these small amounts of normal cystic fibrosis transmembrane receptor protein in these cystic fibrosis patients is likely to be responsible for the milder severity of disease and a better life expectancy.

KEYWORDS: Cystic fibrosis, genotype–phenotype, mild allele, 3849+10kbC->T, 2789+5G->A

Cystic fibrosis (CF) is the most common inherited disorder in Caucasian populations. It is characterised by a chronic and progressive obstructive lung disease, pancreatic insufficiency and high sweat electrolyte levels. Despite being a monogenic disease, CF appears to be very heterogeneous. Indeed, since the cystic fibrosis transmembrane conductance regulator (CFTR) gene was cloned in 1989, >1,300 mutations have been described [1–3]. Moreover, the distribution of these CFTR mutations ranges widely between countries and/or ethnic groups [4].

Several attempts have been made to correlate the phenotype of CF disease with the genotype among patients sharing the same mutations [5–8]. However, since most of the mutations have a low frequency, multi-centre studies are needed to investigate their relationship with CF phenotype. The French CF registry, which annually performs a survey of the patients followed in French CF centres, offers such an opportunity [9]. This study reports the results of a genotype–phenotype correlation analysis for two rare and mild mutations (3849+10kbC->T and 2789+5G->A).

MATERIALS AND METHODS

Since 1992, the French CF registry has collected and analysed data from most of the CF patients regularly seen in CF care centres in France. It is based on a yearly questionnaire that collects demographic, clinical and social data for every CF patient seen during that period. Consent to collect and analyse the data was obtained from the adult patients or the children's parents, at first inclusion in the registry [9].

The first step of the analysis was to extract genotypic data on all the CF patients carrying at least one of the 3849+10kbC->T or 2789+5G->A alleles, who attended a participating care centre anytime between 1992 and 2002.

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Secondly, the available data were extracted for all patients who were compound heterozygous for 3849+10kbC->T/ΔF508 or 2789+5G->A/ΔF508, as previously described [10–12]. This analysis was based on a multi-centre study, with each CF centre being responsible for its own measurements and with the knowledge that patients from a same centre are tested with the same equipment. Each patient was matched to a patient homozygous for the ΔF508 mutation, of same sex and age (±1 yr), having consulted at the same CF care centre. All the data were obtained from the 2000 investigation, except for clinical events that were compiled over the previous 7 yrs (1994–2000).

The mean and median ages on January 1, 2001, and at the time of diagnosis were exact ages expressed as years ± SD. The age at diagnosis was defined as the age at the time when, based on the clinical and biochemical results and the clinical evolution, the diagnosis became evident. Patients diagnosed following antenatal diagnosis and/or neonatal screening were excluded from the calculation. The sweat chloride concentration was measured at the time of diagnosis in the CF care centre and expressed as $mEq \cdot L^{-1}$. A sweat chloride concentration >60 $mEq \cdot L^{-1}$ was considered positive [13]. The pulmonary function and physical status were measured in each CF centre. The pulmonary status was assessed by tests of forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1). The measures were expressed as a percentage of predicted values for sex, age and height according to standardised tables [14]. The Z-scores of weight or height according to sex and age were calculated, using the French population as a reference [15]. A negative Z-score is synonymous with growth retardation. The body mass index (BMI) was also calculated for each patient and expressed in $kg \cdot m^{-2}$, the normal values being included between 20 and 25. A faecal fat-balance study was performed to evaluate the exocrine pancreatic status. A steatocrit test or elastase measurement was also performed. The status at diagnosis consisted of symptoms that prompted the parents or the individuals to consult a physician. The occurrence of several common complications of CF, including liver cirrhosis, diabetes mellitus, distal intestinal obstructive syndrome, recurrent pancreatitis, rectal prolapse or nasal polyposis was used to define the clinical status since 1994.

Descriptive data, expressed as mean values with indication of SD, were analysed through an ANOVA for parametric variables. Categorical variables, expressed as the number of patients in each cell, were compared using the H Kruskal-Wallis test (equivalent to Chi-squared test) for two groups as being nonparametric variables. The significant level was set at $p \leqslant 0.05$.

RESULTS

Since the creation of the French CF registry in 1992, 39 CF patients (23 males, 16 females) carrying at least one 3849+10kbC->T allele have been registered (table 1). During the 1992–2002 period, one female bearing the 3849+10kbC->T/ΔF508 genotype was lost to follow-up at the age of 28 yrs, and three patients (two females and one male) died of respiratory problems at a mean age of 35.4 yrs. The genotype was fully identified for 38 out of the 39 patients (table 1). Two genotypes accounted for almost 80% of the patients,

TABLE 1 Genotypes identified among cystic fibrosis patients sharing the 3849+10kbC->T or the 2789+5G->A mutation

Genotypes	3849+10kbC->T	2789+5G->A
ΔI507		2
ΔF508	27	61
1525-1G->A	1	
1717-1G>A		1
2183AA>G		3
3129del4		1
3659delC		1
G542X	4	6
G551D		1
G970R		2
G1244E	2	
L558S		1
M1V	1	
N1303K		1
R347P	1	
R553X	1	1
R1066C		1
S1251N	1	
Unknown	1	6
Total	39	88

3849+10kbC->T/ΔF508 (n=27, 69.2%) and 3849+10kbC->T/G542X (n=4, 10.3%), and two siblings shared the G1244E allele (5.2%). A total of 36 patients were alive on January 1, 2003. The mean and median ages of the living patients were 22.3 and 23.3 yrs, respectively.

Sixteen (10 males, six females) out of the 27 patients sharing the 3849+10kbC->T/ΔF508 genotype were seen at least once in 2000 and were included in the genotype–phenotype study (table 2). The mean age was similar in both groups ($p>0.05$). Ten (62.5%) 3849+10kbC->T/ΔF508 patients were older than 15 yrs. None had died during the study period. A 3849+10kbC->T/ΔF508 patient received a bipulmonary transplant, while another aged 23 yrs was on the transplantation waiting list.

The mean age at diagnosis was significantly higher among the 3849+10kbC->T/ΔF508 patients than among those homozygous for the ΔF508 mutation (12.7±9.6 *versus* 3.1±5.1 yrs, $p=0.002$). The mean sweat chloride concentration was close to normal values in the compound heterozygote group, whereas it was much higher in the ΔF508 homozygote group (67.9 *versus* 110.4, $p<0.0001$). All 3849+10kbC->T/ΔF508 patients but one had respiratory problems at the time of diagnosis, while the ΔF508 homozygotes were more likely to have intestinal problems, including four patients with meconium ileus *versus* none among the compound heterozygotes ($p=0.03$).

The mean anthropometric values were similar in both groups; the mean BMI was under the lower normal bracket. Although only borderline significant, lung function was definitely better in the 3849+10kbC->T/ΔF508 group (FEV_1 83.0% and FVC 91.6% pred) than in the ΔF508 homozygote group (FEV_1 59.9%

TABLE 2 Characteristics of the patients compound heterozygous for the 3849+10kbC->T/ΔF508 mutations, compared to homozygotes for the ΔF508 mutation

	3849+10kbC->T/ΔF508	ΔF508/ΔF508	p-values
Sex males/females	10/6	10/6	
Age on January 01 2001 yrs			
Mean±SD	19.6±10.2	19.6±10.2	NS
Median	19.5	19.0	
Range	5–47	5–47	
Age at diagnosis yrs#			
Mean±SD	12.7±9.6	3.1±5.1	0.002
Median	11.8	0.8	
Range	0.6–34.0	0.0–16.8	
Sweat chloride conc. mEq·L^{-1}			
Mean±SD	67.9±19.8	110.4±17.4	<0.0001
Median	67.0	116.5	
Range	45.0–95.0	77.0–135.0	
Pancreatic insufficiency %	46.6	100.0	0.002
Sputum cultures			
H. influenzae	5	5	NS
S. aureus	10	8	NS
P. aeruginosa	16	8	NS
Status at diagnosis			
Family history	4	2	NS
Meconium ileus	0	4	0.03
Diarrhoea	1	6	0.03
Respiratory symptoms	15	9	0.01
Physical status			
Height Z-score mean±SD	-0.270±0.660	-0.850±1.412	NS
Weight Z-score mean±SD	-0.540±1.112	-1.214±1.583	NS
BMI kg·m^{-2} mean±SD	16.28±3.26	16.11±3.00	NS
Lung function % pred			
FEV1 mean±SD (median)	83.04±12.08 (81.0)	59.86±21.11 (64.3)	0.069
FVC mean±SD (median)	91.60±8.19 (93.4)	76.96±20.80 (74.2)	0.082
Clinical events since 1994			
No morbidity	8	3	0.01
Liver cirrhosis	0	1	NS
Haemoptysis	4	1	0.069
Nasal polyposis	2	2	NS
Pregnancy/paternity	5	0	0.01

Conc.: concentration; *H. influenzae*: *Haemophilus influenzae*; *S. aureus*: *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa*; BMI: body mass index; FEV1: forced expiratory volume in one second; FVC: forced vital capacity; NS: nonsignificant. #: neonatal screening and antenatal diagnosis excluded.

and FVC 76.9% pred). No difference was found in the prevalence of pathogens between both groups (p>0.05).

Pancreatic insufficiency was present in seven out of the 15 (46.6%) 3849+10kbC->T/ΔF508 patients for whom the status was known, while all 16 ΔF508/ΔF508 patients were pancreatic insufficient (p=0.02). Morbidity was lower among the 3849+10kbC->T/ΔF508 patients (p<0.01), with no patient with liver cirrhosis or diabetes mellitus. Five out of the 16 compound heterozygotes had had children *versus* none among their matched peers.

Since the French CF registry was implemented in 1992, 88 CF patients (44 males, 44 females) bearing the 2789+5G->A mutation have been registered (table 1). During the 1992–2002 period, four adult patients (two males and two females) were lost to follow-up, and two (one male and one female) died at 42 and 37 yrs, respectively. The genotype was fully identified for 82 out of the 88 patients. Three genotypes accounted for almost 80% of the patients: 2789+5G->A/ΔF508 (n=61, 69.3%), 2789+5G->A/G542X (n=6, 6.8%) and 2789+5G->A/2183AA->G (n=3, 3.4%). A total of 82 patients (93.2%) were alive on January 1, 2003. The mean and median ages of the living patients were 25.1 and 23.7 yrs, respectively.

Thirty-four patients (16 males, 18 females) carrying the 2789+5G->A/ΔF508 genotype and seen in 2000 were included in the genotype–phenotype study (table 3). The mean age was similar in both groups (26.26±11.3 yrs), 25 of them (73.5%) being older than 15 yrs. No patient died during the study period.

The mean age at diagnosis was significantly higher among the 2789+5G->A/ΔF508 patients than among those homozygous for the ΔF508 mutation (16.6±12.7 *versus* 4.5±8.9 yrs, p=0.0001). The mean sweat chloride concentration was high in both groups, with no statistically significant difference. The 2789+5G->A/ΔF508 patients had very few gastrointestinal symptoms at the time of diagnosis. None had meconium ileus (p=0.01) and only two had malnutrition or diarrhoea, respectively (p=0.03 and p=0.001). Conversely, they had an increased frequency of nasal polyposis compared with the ΔF508 homozygotes (p=0.02).

The anthropometric values were better among the compound heterozygotes, with the mean Z-score for weight and the BMI within normal ranges. Lung function was also better in the 2789+5G->A/ΔF508 group; however, only the difference in the mean FEV1 between both groups reached statistical significance (p=0.03). The median values for FEV1 and FVC were higher among those patients carrying the 2789+5G->A mutation (80.8 *versus* 57.9% and 94.8% *versus* 80.8%, respectively).

The sputum culture results showed significant differences between those compound heterozygotes for the 2789+5G->A mutation and those homozygous for the ΔF508 allele, the former having an increased prevalence of *Haemophilus influenzae* (p=0.002) and *Staphyloccocus aureus* (p=0.004), but a lower prevalence of *Pseudomonas aeruginosa* (p=0.00005).

Pancreatic insufficiency was present in 19 out of the 32 (59.4 %) 2789+5G->A/ΔF508 patients for whom the status was known, while 32 out of 33 (97.0%) ΔF508/ΔF508 patients were pancreatic insufficient (p=0.002). Morbidity was also lower, with no patient having liver cirrhosis or diabetes mellitus.

DISCUSSION

As first described by HIGHSMITH *et al*. [16] in 1994, the 3849+10kbC->T mutation was identified in 13 patients with chronic pulmonary disease but normal sweat chloride values. It is due to the insertion of 10 kb in intron 19, inducing a C to T

TABLE 3 Characteristics of the patients compound heterozygous for the 2789+5G->A/ΔF508 mutations, compared with homozygotes for the ΔF508 mutation

	2789+5G->A/ΔF508	ΔF508/ΔF508	p-values
Sex males/females	16/18	16/18	
Age on January 01 2001 yrs			
Mean±SD	26.3±11.4	26.2±11.3	NS
Median	26.0	26.0	
Range	5–51	5–47	
Age at diagnosis yrs#			
Mean±SD	16.6±12.7	4.5±8.9	0.0001
Median	15.8	0.8	
Range	0.2–46.5	0.08–35.3	
Sweat chloride conc. mEq·L^{-1}			
Mean±SD	104.4±25.1	115.3±22.5	NS
Median	106.0	119.0	
Range	62.0–150.0	77.0–155.0	
Pancreatic insufficiency %	59.4	97.0	0.002
Sputum cultures			
H. influenzae	13	3	0.002
S. aureus	26	17	0.004
P. aeruginosa	8	25	0.00005
Status at diagnosis			
Meconium ileus	0	5	0.01
Malnutrition	2	8	0.03
Diarrhoea	2	13	0.001
Nasal polyposis	5	0	0.02
Respiratory symptoms	20	15	NS
Physical status			
Height Z-score mean±SD	-0.19±0.97	-0.60±1.07	NS
Weight Z-score mean±SD	0.36±2.01	-0.73±1.61	0.02
BMI kg·m^{-2} mean±SD value	20.2±3.5	18.8±2.7	NS
Lung function % pred			
FEV_1 mean±SD (median)	75.38±29.69 (80.8)	59.06±24.87 (57.9)	0.03
FVC mean±SD (median)	89.03±27.07 (94.8)	78.03±22.80 (80.8)	NS
Clinical events since 1994			
Liver cirrhosis	0	6	0.016
Diabetes mellitus	0	2	NS
DIOS	0	2	NS
Nasal polyposis	8	8	NS

Conc.: concentration; *H. influenzae*: *Haemophilus influenzae*; *S. aureus*: *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa*; BMI: body mass index; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; DIOS: distal intestinal obstructive syndrome; NS: nonsignificant. #: neonatal screening and antenatal diagnosis excluded.

change and leading to the creation of an alternative splice acceptor site.

The Cystic Fibrosis database reports that the worldwide frequency of the 3849+10kbC->T mutation is close to 0.2% of all CF chromosomes [4]. In Europe, its frequency is 0.15% of CF chromosomes, although it is more common among Jews from eastern Europe and in the Polish population (~4% of CF alleles) [17–20]. A higher frequency (~2%) is also reported in Hispanic and Native American patients [21, 22]. The 3849+10kbC->T mutation was found to be associated with four microsatellite haplotypes in Europe, while a fifth haplotype was identified in the Native American population, consistent with the hypothesis of a recurrent mutation [22, 23].

In the population presented here, 39.3% of the patients, for whom the birthplace was registered, were from the north-east region of France (Nord-Pas-de-Calais and Lorraine), where ~300,000 Polish individuals settled between 1921 and 1938, and where, nowadays, one in eight inhabitants has Polish roots. Unfortunately, microsatellite haplotypes of patients are not available in the French CF registry, in order to check the homogeneity of the 3849+10kbC->T mutation in this population.

AUGARTEN *et al*. [24] previously investigated 15 CF patients carrying the 3849+10kbC->T allele and compared their clinical status with that of an unmatched group of 57 patients who were compound heterozygous or homozygous for the ΔF508 or W1282X mutations. Patients with the 3849+10kbC->T mutation were older and had been diagnosed at a more advanced age. They were more likely to be pancreatic sufficient (PS), and have no diabetes mellitus or liver cirrhosis. They were in a better nutritional but not pulmonary state.

GILBERT *et al*. [25] also reported the clinical spectrum of CF among 14 patients who were compound heterozygous or homozygous for the 3849+10kbC->T mutation, without matching them to ΔF508 homozygotes. The age at diagnosis ranged 2–32 yrs. Half of the patients were PS, the sweat chloride concentration being within normal values in seven patients. Lung disease varied from mild to severe.

In 1995, STERN *et al*. [26] reported eight patients who were compound heterozygous for the 3849+10kbC->T mutation. All had normal or borderline sweat chloride values, were PS, but had a variable pulmonary disease ranging from mild to severe. FELDMANN *et al*. [27] collected data on CF patients with normal or borderline sweat chloride levels and two CFTR mutated alleles. One fourth carried the 3849+10kbC->T allele, which was the most frequent allele observed in the study. None had sweat test values >60 mmol·L^{-1} (range 18–55.5), but all had pulmonary symptoms. Five of them had been diagnosed at age 20 yrs or over.

The 2789+5G->A allele was also first described by HIGHSMITH *et al*. [28]. A G to A substitution was observed at nucleotide 2,789 in a splice donor site (intron 14b), leading to an mRNA splicing, class 5 mutation. Apart from the consanguineous family in which the mutation was first described, no clinical evaluation of the 2789+5G->A/ΔF508 genotype was found in the literature. This mutation accounts for 0.1% of the CF chromosomes worldwide. In Europe, ESTIVILL *et al*. [17] found a high frequency in the south of Greece (4.5% of chromosomes), whereas, in France, CLAUSTRES *et al*. [29] found it to be more frequent in the north (2.87%) than near the Mediterranean coast. In the current population, 30 out of the 65 patients (46.1%) for whom the birth place was known were from the north-east region of France.

This appears to be the first study in which 3849+10kbC->T/ΔF508 or 2789+5G->A/ΔF508 patients were matched to ΔF508

homozygotes. Both groups were associated with delayed diagnosis and a higher frequency of pancreatic sufficiency. Although they had high sweat chloride values, patients carrying the 2789+5G->A allele had a milder phenotype, characterised by better anthropometric and lung function measures, less colonisation with *P. aeruginosa* and, probably, a higher life expectancy. Patients carrying the 3849+10kbC->T mutation also appeared to have a milder phenotype, with better anthropometric and lung function measures.

Both populations (3849+10kbC->T/ΔF508 and 2789+5G->A/ΔF508) also appeared to differ from the other patients included in the French CF registry. Indeed, the mean and median ages at the time of diagnosis of the overall population were, respectively, 2.8 yrs and 4 months; the mean and median ages of the registered population were 14.9 and 13 yrs. Moreover, the proportion of the adults with the 3849+10kbC->T/ΔF508 or 2789+5G->A/ΔF508 genotype was increased: >50% were 18 yrs, compared with 35.5% of the patients included in the registry [30]. The results presented here also show that the 3849+10kbC->T/ΔF508 and 2789+5G->A/ΔF508 populations are closed to CF adults with delayed diagnosis [31–33]. In fact, the 3849+10kbC->T/ΔF508 or 2789+5G->A/ΔF508 genotype can be included in the adult group defined by HUBERT *et al*. [8] as patients with expected partly functional CFTR corresponding to at least one ''mild'' mutation. In these patients, the age at diagnosis is >20 yrs with no meconium ileus, less pancreatic insufficiency but respiratory problems.

Several differences, although large, with the ΔF508 homozygotes did not reach statistical significance. This could be due to the small numbers of patients included in this multi-centre study or to the high variation sometimes observed within the ΔF508 homozygous group. One way to overcome this last problem would have been to partner each compound heterozygous patient to three ΔF508/ΔF508 patients matched for sex, age and care centre, and to perform the statistical analysis on the ''mean ΔF508 homozygote population''. Unfortunately, this was impossible, due to the difficulty of finding more than one peer, especially in older age groups (the oldest 2789+5G->A/ΔF508 patient included in the present study was 51 yrs).

Despite these difficulties, it can be concluded that both 3849+10kbC->T and 2789+5G->A mutations are associated with a milder course of cystic fibrosis disease. The reasons for such moderate disease can probably be found in the nature of the mutations. The 3849+10kbC->T and 2789+5G->A alleles are splice site mutations leading to abnormal mRNA; however, small amounts of normally spliced transcripts are also detected (4–8% of that found in normal individuals) [16, 28]. The presence of these small amounts of normal cystic fibrosis transmembrane receptor protein in the cystic fibrosis patients carrying the 3849+10kbC->T or 2789+5G->A mutation is likely to be responsible for a milder disease and a better life expectancy.

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