

ONLINE MUTATION REPORT

Splice mutation 1811+1.6kbA>G causes severe cystic fibrosis with pancreatic insufficiency: report of 11 compound heterozygous and two homozygous patients

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Cystic fibrosis (CF, OMIM 219700) is one of the most common severe hereditary diseases occurring in white populations. It is characterised by considerable heterogeneity in the mutational spectrum and a diverse phenotypic presentation,¹ which justifies extensive studies on the correlation between the cystic fibrosis transmembrane regulator (*CFTR*) genotype and the CF phenotype. Genotype-phenotype data are available especially for common genotypes,² but information remains scarce or lacking for genotypes of compound heterozygotes with a rare mutation and homozygotes for a rare mutation.³

Among rare *CFTR* gene mutations, 1811+1.6kbA>G is practically absent in CF patients from other parts of France, but it is not rare in patients genotyped in the south west of France, occurring in fourth place after F508del, G542X, and N1303K. Its frequency is 3.4%⁴ and higher than 5% with reference to the number of CF chromosomes with a south western geographical origin.⁵ The mutation 1811+1.6kbA>G, located in intron 11 of the *CFTR* gene, is (with the mutation 3849+10kbC>T) one of the only two splicing mutations of the *CFTR* gene that generates a new exon (1811+1.6kbA>G creates a donor site, 3849+10kbC>T an acceptor site). Furthermore, it was previously ranked by Chillon *et al*⁶ among class I mutations responsible for defective *CFTR* protein production and a severe CF phenotype. However, because it allows a reduced synthesis of normal protein, the mutation 1811+1.6kbA>G has also been reported in class V.^{7,8} According to the recent classification proposed by Welsh *et al*,⁹ the mutation 1811+1.6kbA>G now belongs definitely to the "new" class I.

The present report aims to study as well as possible the clinical severity of the mutation 1811+1.6kbA>G. The clinical data from 13 CF patients originating from the south west region of France and bearing 1811+1.6kbA>G (11 compound heterozygotes and two homozygotes) were collected.

PATIENTS AND METHODS

The clinical characteristics of 13 patients carrying the mutation 1811+1.6kbA>G (table 1) were obtained from various hospitals in the Aquitaine and Midi-Pyrénées regions of France.

The parameters recorded were: (1) for all patients, the current age, age of diagnosis, clinical diagnosis, time of follow up, sweat chloride concentration, colonisation by *Pseudomonas aeruginosa* (three consecutive positive cultures), height and weight, pancreatic exocrine function (mainly assessed clinically and sometimes with the faecal elastase value); (2) only for patients older than 5, the maximal expired volume per second (FEV1) and the forced vital capacity (FVC); (3) for the majority of patients, the Brasfield radiological score and the Schwachman score.

Genotyping of *CFTR* gene mutations was carried out on DNA extracted from leucocytes in a two step procedure. First, the most common mutations in the French population were investigated by multiplex PCR amplification and subsequent reverse

Key points

- The splice mutation 1811+1.6kbA>G of the *CFTR* gene is the fourth most frequent mutation (after F508del, G542X, and N1303K) in CF patients from the south west of France.
- To increase knowledge of its severity, the clinical data from 13 CF patients bearing this mutation (11 compound heterozygotes and two homozygotes) were collected.
- They clearly show that the class I mutation 1811+1.6kbA>G is responsible for a typical cystic fibrosis phenotype with pancreatic insufficiency.

dot blot hybridisation by using INNO-LiPA CF2, then INNO-LiPA *CFTR*12 and *CFTR*17+Tn assays (Innogenetics, Zwijnaarde, Belgium). When one or two mutations were still to be found, an exhaustive search for uncommon mutations was done by denaturing gradient gel electrophoresis (DGGE)¹⁰ and automatic sequencing with Dye Terminators on ABI PRISM 377 (Applied Biosystems). For the first patients investigated, the mutation 1811+1.6kbA>G was detected by automatic sequencing (ABI PRISM 377, Applied Biosystems) of a specific region of intron 11 according to a procedure used in our laboratory (unpublished data). Then the search for the mutation 1811+1.6kbA>G was done directly by PCR of intron 11 and *FokI* digestion⁶ simultaneously with the reverse dot blot step.

RESULTS

The results of genotyping the 13 CF patients carrying the mutation 1811+1.6kbA>G were: two homozygotes for 1811+1.6kbA>G/1811+1.6kbA>G and 11 compound heterozygotes for 1811+1.6kbA>G and for another *CFTR* mutation, that is, F508del (n=6), N1303K (n=2), G542X (n=1), 2183 AA>G (n=1), and W1063X (n=1). No complex alleles were found among the 13 patients with 1811+1.6kbA>G.

Table 1 lists the clinical characteristics of all the patients with the mutation 1811+1.6kbA>G. They are very similar to patients homozygous for F508del or compound heterozygotes for two severe *CFTR* mutations. All presented a typical form of cystic fibrosis that was diagnosed early (nine out of 13 before the age of 1 year), then confirmed by a positive sweat test; for the 13 patients, the chloride mean (SD) and the median (min-max) values are respectively 88 (SD 22) and 82 (57-120) mmol/l. Three patients (23%) had neonatal meconium ileus. In 10 cases, there was colonisation by *Pseudomonas aeruginosa* and in 11 patients growth delay was observed. The obstructive syndrome of the lungs was severe and always accompanied by pancreatic insufficiency (PI).

Table 1 Characteristics of the 13 CF patients with mutation 1811+1.6kbA>G

Genotype*	Patient No												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Sex	M	M	F	M	F	F	M	F	M	F	F	M	M
Current age (y)	8.3	4.9	12.5	2.2	24.2	2.2	22.1	18.1	1.3	10.6	23.5†	16†	14
Age at diagnosis (y)	0.3	0	0.1	0	0.5	1	10	4	0	3	0.1	6	0.3
First symptoms	Digestive	Meconium ileus	Neonatal screening	Meconium ileus	Pulmonary, hypotrophy	Pulmonary, hypotrophy	Digestive	Pulmonary, digestive, hypotrophy	Meconium ileus	Pulmonary, digestive	CF family history	Pulmonary	Pulmonary, digestive
Sweat chloride (mmol/l)	120	70	77	90	82	115	70	120	57	90	77	63	108
<i>P. aeruginosa</i> colonisation	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Weight (SD)	-2	-3	-0.5	-1	-2	-2	0.5	-3	0.5	-2	0	-3	-3
Height (SD)	-2	-1.5	-0.5	0	-2	0	1	-2.5	1.5	-2	0	-3	-3
FEV ₁ (% predicted)	111	13	104	2	35	35	33	54	70	70	68	30	40
FVC (% predicted)	102	9	102	2	45	45	58	67	70	90	40	21	3
Brasfield score	9	20	5	2	80	18	20	16	0	10	12	21	3
Shwachman score	9	20	90	16	80	50	55	60	95	80	55	35	15
Elastase (µg/g)†	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Other clinical features	Biliary lithiasis	Gastro-oesophageal reflux		Dehydration	Biliary lithiasis, heart-lung transplantation	Type 2 diabetes	Malnutrition	Yes	Yes	Yes	Severe malnutrition	Yes	Congenital encephalopathy

*1811 = 1811+1.6kbA>G; †decreased; ‡normal values: > 200 µg/g

DISCUSSION

Up to 2000, we used to consider five classes of *CFTR* mutations defined according to the functionality of the CFTR protein.⁷⁻¹¹ They correspond either to an absence of protein production (class I), or a trafficking defect (class II), or defective regulation (class III), or defective conductance (class IV), or a reduced level of normal messenger RNA or protein (class V). A new class of *CFTR* mutation (class VI) has recently been proposed leading to a decreased stability of the protein.¹³ However, recently, Welsh *et al*⁹ have slightly modified the classification of *CFTR* gene mutations. They defined a new class I corresponding to either defective (previous class I) or reduced (previous class V) protein production. Classes II, III, and IV remain the same. What was called class VI before may now be considered as the “new” class V.

The genotype-phenotype relationships of some *CFTR* mutations are easy to classify. This is the case for nonsense and frameshift mutations always associated with severe CF with PI: they come into class I (for instance, G542X) when they lead to an absence of functional CFTR and into the “new” class V (for instance, Q1412X) when they cause the presence of a functional but unstable CFTR at the apical membrane. A severe CF presentation correlates also with class II mutations such as the amino acid deletion F508del.

There is considerable heterogeneity in missense mutations. Some of them are always responsible for a unique phenotype that can be either CF-PI (for instance, the case of N1303K in class II, G551D in class III, and R1066C in class IV), or CF-PS (for instance, the case of G551S in class III) or CBVAD for D1152H (class IV). Other missense mutations show considerable phenotypic variation, such as CF-PI or CF-PS with R334W, CF-PS or congenital bilateral absence of the vas deferens (CVABD) with R117H (class IV). Splicing mutations also have a variable impact on the phenotype depending on the position of the nucleotide change at the splice site. When one of the two nucleotides beginning or ending introns is concerned (for instance with 621+1G>T or 1717-1G>A), the mutation is in class I and CF is severe with PI.¹⁴ When the mutation occurs further from the exon-intron junction, the phenotype is less severe: CF-PS in the case of 2789+5G>A (class IV) and either CF-PS or CBAVD when the 5T allele is present.

Particular emphasis should be given to alternative splice mutations in class I that create a new exon. They result in reduced expression of functional CFTR channels in the apical membrane. They allow the presence of a small quantity of normal residual CFTR on which depends the degree of severity. These mutations, 3849+10kbC>T (intron 19) and 1811+1.6kbA>G (intron 11), are only two of the 1000 mutations reported so far (The Cystic Fibrosis Genetic Analysis Consortium, CFGAC: <http://genet.sickkids.on.ca>). Phenotypic variability has been largely reported for 3849+10kbC>T,^{15,16} but very few patients with 1811+1.6kbA>G, only compound heterozygotes, have been reported so far. Our results concerning 13 patients with the mutation 1811+1.6kbA>G including two homozygotes are in good agreement with previous data⁹ and confirm that this CF mutation is a severe one; all 13 patients had respiratory and digestive disease as shown by their clinical characteristics and by comparison with CF patients homozygous for F508del. The severity of the disease is similar to the one resulting from F508del with even more severe growth retardation with 1811+1.6kbA>G. The clinical features of the homozygotes 1811+1.6kbA>G/1811+1.6kbA>G (patients 1 and 2 in table 1) show that the phenotype remains severe, whereas one might expect a moderation of the phenotype because of twice as much theoretical amount of functional CFTR than in patients with one dose of mutation 1811+1.6kbA>G.

In conclusion, we suggest that the CF-PI mutation 1811+1.6kbA>G ranks among the mutations of class I with

reduced protein production as an exception, since all the other class I mutations of this kind cause considerable phenotypic variability in CF disease and not limited to CF-PI. It is not known why the phenotype is not attenuated despite the presence of higher amounts of normal CFTR protein in patients homozygous for 1811+1.6kbA>G. Further investigations including in vitro expression of the mutation are needed for a better understanding of the mechanism of this CFTR mutation.

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