Journal of Cystic Fibrosis 7 (2008) 292–294

www.elsevier.com/locate/jcf

Diagnosis of atypical CF: A case-report to reflect

F. Alghisi [a], A. Angioni [b], A.C. Tomaiuolo [b], M.R. D'Apice [c], S. Bella [a], G. Novelli [c], V. Lucidi [a,*]

[a] *Unit of Cystic Fibrosis, Bambino Gesù Children's Hospital—Rome, Italy*
[b] *Laboratory of Cytogenetics and Molecular Genetics, Bambino Gesù Children's Hospital—Rome, Italy*
[c] *Department of Biopathology and Diagnostic Imaging, Tor Vergata University—Rome, Italy*

Received 1 October 2007; received in revised form 12 November 2007; accepted 13 November 2007
Available online 3 January 2008

Abstract

Non-classic Cystic Fibrosis (CF) still represents a difficult entity to diagnose. We present a case of two sisters affected by mild pulmonary symptoms started at puberty, carriers of the F508del mutation associated with the T5TG13 combination. We discuss the clinical utility of TG repeat testing in individuals carrying the T5 variant. Furthermore, this case-report leads to reflect on the natural history of CF and the correct management of its atypical forms.

Keywords: Atypical Cystic Fibrosis; Diagnosis; CFTR; IVS8-5T; Poly-TG(m)

1. Introduction

Diagnosis of classic Cystic Fibrosis (CF; OMIM #219700) is easy because of multiple organ involvement and supported by a positive sweat test. On the contrary, non-classic CF still represents a difficult entity to define for clinicians, especially at the early stage of the disease, due to its unusual presentation and/or late onset of symptoms. Moreover, the sweat test may show border-line or normal values and the diagnosis is mainly based on clinical features and follow-up [1,2]. Genetic analysis of the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene may identify one severe or mild mutation *in trans* with a 5 thymidines (T5) sequence within the intron 8 Splice Variant (IVS8) region. T5 is responsible for an exacerbated skipping of exon 9, decreasing the functional product levels. Its phenotypic expression is variable [3] and influenced by another adjacent polymorphic region, constituted by 9 to 13 TG repeats. The relevance of TG repeats in influencing the T5 expression has been demonstrated by Groman et al. [4] who showed that T5TG12 and T5TG13 were the most frequent combinations in patients affected by non-classic CF. In particular, T5TG13 was found only in affected subjects. Another study [5] confirmed that exon 9 splicing efficiency decreases as the number of TG repeats increases, suggesting that altered splicing depended on changes in RNA secondary structures.

1.1. Case report

A 23-year-old female, weight 58 Kg, height 176 cm, BMI 18.7 Kg/m^2, with negative family history of CF, referred to our centre because of chronic cough, previously treated unsuccessfully for asthmatic bronchitis. She suffered from three episodes of pneumonia in the previous 5 years. Head and chest CT, after the last pulmonary infection, revealed bilateral bronchiectasis and pansinusitis. This finding suggested that the patient could be affected by Kartagener Syndrome, successively excluded by a ventilatory scintiscan that did not show a muco-ciliary clearance delay. In our centre this patient was investigated to ascertain the presence of CF; two different sweat tests showed border-line chloride levels (46 and 51 mmol/L, respectively). Therefore, it was performed genotype testing for the most common *CFTR* gene mutations that revealed the presence of F508del *in trans* with the T5TG13 and *in cis* with the T9TG10 combinations. DHPLC screening was also evaluated but it did not reveal further *CFTR* mutations. Additionally, no gross

* Corresponding author. Piazza S. Onofrio 4, 00165, Rome, Italy. Tel./fax: +39 06 6859 2045.
E-mail address: lucidi@opbg.net (V. Lucidi).

1569-1993/$ - see front matter © 2007 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jcf.2007.11.002

genomic rearrangements were found by means of MLPA method. Pulmonary function test showed a mild lung disease (FEV1 = 3.36 L/s, 89.2% predicted). At follow-up the patient's sputum culture was positive for *Pseudomonas aeruginosa* and *Staphylococcus aureus*; these infections were treated with specific antibiotics selected on the basis of the antibiogram. The fecal elastase test resulted negative (>500 μg/g feces). These data suggested a diagnosis of atypical CF without pancreatic insufficiency.

We also studied the patient' sister, aged 18 years. At presentation: weight 57 Kg, height 166 cm, BMI 20.7 Kg/m^2. She complained of mild symptoms (recurrent cough) indicating a pulmonary involvement. She was pancreatic-sufficient and did not refer any abdominal disorder. Chloride level at two different sweat tests was border-line (34 and 35 mmol/L, respectively) and the genetic investigation showed the same pattern of her sister consisting in F508del *in trans* with the T5TG13 and *in cis* with the T9TG10 combinations. The patient underwent a chest CT which showed rare bronchiectasis in the posterior segment of the lower lobe of both lungs. Spirometry was normal, with a FEV1 of 3.55 L/s (118.1% predicted), and the sputum culture resulted sterile.

To complete the genetic evaluation, both asymptomatic parents were tested: the mother resulted homozygous for the T5 allele and carrier of a TG12TG13 combination; the father resulted heterozygous for the F508del mutation, associated with the T7 T9 combination.

2. Discussion

We report a case regarding two sisters carriers of the F508del mutation associated with the T5TG13 combination. Both present mild pulmonary symptoms started at puberty, bronchiectasis, pancreatic sufficiency and border-line chloride values revealed by the sweat test. However, they differ because the elder patient has more evident bronchiectasis, and she also presents pansinusitis, a positive sputum culture and a slightly reduced FEV1. Our report confirms that the presence of T5 allele, *in trans* with a severe *CFTR* mutation, is associated with non-classic CF and that TG13 variant acts as a real mild mutation, enhancing the T5 penetrance and determining the onset of a mild symptomatology in all patients. Thus, this observation confirms the clinical utility of TG repeat testing in individuals carrying the T5 variant, although it can not explain the whole clinical variability seen in CF, as in our two patients of our report. Phenotype could be influenced by other factors, such as gender. Groman et al. showed that carriers of the T5TG12 or T5TG13 alleles *in trans* with a *CFTR* mutation are at higher risk of developing non-classic CF, if females, and congenital bilateral absence of vas deferens, if males [4]. Similar differences have also been described in patients carrying the F508del/R347H compound heterozygosis [6]. Furthermore, a gender gap exists in CF about survival, with females having a significantly higher mortality than males [7], persisting despite a consistent improvement in survival rates through the years [8]. The reason of these differences is still unknown; however, some authors have suggested that mutations in the Y chromosome could mitigate the CF phenotype in males toward less severe manifestations, as an evolutionary mechanism preserving fertility [9]; alternatively, it is possible that *CFTR* gene reflects a tissue-specific expression according to gender [10]. However, age difference between the two patients appears the most likely cause of their phenotypic variability. In fact, their age difference is of 5 years, a period of time that could be sufficient to develop an advanced lung disease in the elder sister. Therefore, an earlier diagnosis and a close follow-up could probably avoid or delay the organ deterioration in the younger sister.

The natural history of non-classic CF is poorly understood. Studies on the Bronchoalveolar Lavage (BAL) technique in infants with CF have provided more precise microbiological data than upper airways cultures and relevant information about the presence of inflammation, detected since four weeks of life, even in the absence of infection [11,12]. The use of this invasive approach may be justified in patients with classic CF but it is unlikely applicable to those affected by atypical CF, given their mild symptomatology and good prognosis. It is still unclear when and how atypical CF starts and develops, and what kind of prevention therapy is needed. However, CF has not to be considered an "all or none disease" because chemical and/or genetic CF markers may be asymptomatic for years and they may or may not develop evidence of clinical disease [13]. Therefore, the evidence of a significant lung involvement, as seen in the elder sister, suggests that a prevention therapy should be necessary also in mild, non-classic CF.

Acknowledgements

The study was supported by the Foundation for the Research on Cystic Fibrosis (FFC # 23/2006).

References

[1] Boyle MP. Non-classic Cystic Fibrosis and CFTR-related diseases. Curr Opin Pulm Med 2003 Nov;9(6):498–503.
[2] De Boeck K, Wilschanski M, Castellani C, et al. Cystic fibrosis: terminology and diagnostic algorithms. Thorax 2006;61(7):627–35 [Electronic publication, 2005 Dec 29].
[3] Chillón M, Casals T, Mercier B, et al. Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. N Engl J Med 1995 Jun 1;332(22):1475–80.
[4] Groman JD, Hefferon TW, Casals T, et al. Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign. Am J Hum Genet 2004 Jan;74(1):176–9 [Electronic publication, 2003 Dec 18].
[5] Hefferon TW, Groman JD, Yurk CE, et al. A variable dinucleotide repeat in the CFTR gene contributes to phenotype diversity by forming RNA secondary structures that alter splicing. Proc Natl Acad Sci USA 2004 Mar 9;101(10):3504–9 [Electronic publication, 2004 Mar 1].
[6] Kosztolányi G, Malik N, Rutishauser M. Mild CF in a ΔF508/R347H compound heterozygote woman: does the manifestation of this genotype differ in the two sexes? Clin Genet 1996 Feb;49(2):103–5.
[7] Davis PB. The gender gap in cystic fibrosis survival. J Gend Specif Med 1999 Mar–Apr;2(2):47–51.
[8] Kulich M, Rosenfeld M, Goss CH, et al. Improved survival among young patients with cystic fibrosis. J Pediatr 2003 Jun;142(6):631–6.

[9] Creus M, Vogel W, Zoller H. Of genes and men: the alternative view of sex differences in cystic fibrosis: response to Sims et al. and Milla et al. Diabetes Care 2006 Jan;29(1):179–80.
[10] Xu WM, Shi QX, Chen WY, et al. Cystic fibrosis transmembrane conductance regulator is vital to sperm fertilizing capacity and male fertility. Proc Natl Acad Sci USA 2007 May 22. doi:10.1073/pnas.0609253104.
[11] Ramsey BW, Wentz KR, Smith AL, et al. Predictive value of oropharyngeal cultures for identifying lower airways bacteria in cystic fibrosis patients. Am Rev Respir Dis 1991 Aug;144(2):331–7.
[12] Khan TZ, Wagener JS, Bost T, et al. Early pulmonary inflammation in infants with cystic fibrosis. Am J Respir Crit Care Med 1995 Apr;151(4):1075–82.
[13] Bush A, Wallis C. Time to think again: Cystic Fibrosis is not an “All or None” disease. Pediatr Pulmonol 2000 Aug;30(2):139–44.