



Case Study

Impaired CFTR function in mild cystic fibrosis associated with the S977F/T5TG12 complex allele in trans with F508del mutation

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Abstract

Background: The S977F mutation (c.2930C>T) in the CFTR gene (CFTR/ABCC7) is extremely rare. We describe the case of an adult patient carrying the complex allele S977F/T5TG12 in trans with the F508del mutation. Mild respiratory manifestations arose in adulthood associated with azoospermia, acute pancreatitis, minor hemoptysis and Cl⁻ levels ranging from 40 to 42 mEq/L.

Method: Diagnosis was confirmed by repeated NPD measurements, genetic DHPLC analysis and a recently described functional assay measuring cAMP-dependent cell depolarization in peripheral blood monocytes.

Results: NPD measurements, DHPLC and monocyte functional assay (CF index = -18). Results were consistent with a CF phenotype.

Conclusions: The combined application of DHPLC and NPD analysis in the algorithm for CF diagnosis appears useful for the management of similar cases. In addition, the novel monocyte functional assay might contribute to improve our diagnostic capability, counseling and better treatment of these challenging clinical cases.

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CF is characterized by autosomic recessive inheritance, with an incidence of about 1 in 3000 among Caucasians, and mutations in the CFTR gene as basic defect. The clinical manifestations of CF are extremely varied. To the present day, more than 1900 mutations have been identified in the CFTR gene. Most of them are somewhat rare, making CF diagnosis by genotyping very difficult. Typical CF manifestations include sweat chloride concentrations above 60 mmol/L, sino-pulmonary disease, specific gastrointestinal and/or nutritional abnormalities, salt loss syndrome and male infertility

caused by congenital bilateral absence of vas deferens (CBAVD). CF is typically associated with exocrine pancreatic insufficiency, gradual loss of pulmonary function and lung tissue destruction, and less frequently with sufficient residual pancreatic function and a milder clinical picture [1]. A range of even milder conditions caused by CFTR dysfunction are known as CFTR related disorders [2], and they are associated with limited deterioration over time and clinical manifestations in at least one organ in the presence of normal or borderline sweat chloride values. The lower limit of the borderline range varies between 30 and 40 mmol/L according to age and international guidelines [3,4].

In these subjects diagnosis must be confirmed through additional testing including the search for rare CFTR mutations and *in vivo* evaluation of the CFTR function by NPD measurement.

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Measurement of intestinal currents, as an alternative or in addition to NPD, may also be used [3]. Most of these patients have exocrine pancreatic sufficiency.

Diagnosis of non classical CF [3] is challenging. This is due not only to the variety of manifestations that are the results of the combination of the functional consequences of different classes of CFTR gene mutations together with the effect of the genetic background, including non-CF modifier genes, and of the environmental factors [5].

In the summer of 2007 a 43 year-old male contacted our Center for diagnostic definition, following sweat testing, with chloride levels ranging from 38 to 44 mEq/L and a CT scan showing bilateral bronchiectasis. Other clinical manifestations included azoospermia, acute pancreatitis and minor hemoptysis, allergic bronchopulmonary aspergillosis and chronic lung infection by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Lung function was normal.

Sweat testing was carried out twice at our Center, according to the Gibson and Cooke method (Sweat testing: Sample Collection and Quantitative Analysis — Approved Guideline — Second Edition — The National Committee for Clinical Laboratory Standards (NCCLS) — document C34 A2 [ISBN-56238-407-4]; USA; 2000). The resulting chloride levels were 40 and 42 mEq/L.

An initial analysis was carried on with the aim of finding any of the CFTR mutations that occur most frequently in the population of the regions of northeast Italy, and for large deletions in the CFTR gene, including CFTRdele17a-18 (3120+1kdel8.6kb) and CFTRdele1 (c4_IVS1+69del119bpins299bp). Reverse dot blot (kits provided by InnoLipa, Innogenetics, Belgium) was used. Following the detection of the F508del mutation, D-HPLC and sequencing were performed and results showed the presence of the IVS8-(TG)mTn polymorphic alleles (TG)12T5/(TG)10T9, and of the S977F mutation. It was not possible to test the parents since they live in another region but three sisters were genotyped: one had S977F, IVS8-Tn: T5/T7; one no mutations/variants, IVS8-Tn: T7/T7; the other had F508del, IVS8-Tn: T9/T7. From this we concluded that S977F was in *cis* with the polymorphic allele T5.

In the transmembrane domain 9 the residue 977F is highly conserved among five species [6]. In comparison with L977F, which is more conservative and considered as a polymorphism [6,7] or associated with increased susceptibility to pancreatic ductular obstruction, the rare mutation S977F [8] is less conservative. The presence of this mutation is associated with possible effects on function and consequent clinical manifestations. This prediction is consistent with the case reported in the literature, where a subject carrying the S977F mutation, in addition to a “severe” classic CF-causing mutation on the other allele (CFTR genotype G542X/S977F), presented with clinical evidence of CF [9]. This observation could well be applicable to L997F too. L977F has been associated with CF and a complex allele (R117L,L977F) has recently been described that could account for the variable phenotype [10]. The presence of R117L was excluded in our patient. The case we describe, the S977F allele in *cis* with the polymorphic site TG12T5, allows the possibility that decreased mRNA expression, associated with the TG12T5 allele, participates together with the amino acid replacement, to CFTR functional impairment. In the case

described by Férec et al. the IVS8-Tn genotype was not reported. In the Clinical and Functional Translation of CFTR 2 (CFTR2) worldwide CF database, S977F is reported in 9 patients with 6 of them having F508del in trans. Variable expression or penetrance of this mutation has been demonstrated by clinical and functional analyses (<http://www.genet.sickkids.on.ca/resource/nl/CFnewslet.69.html>). If compared with all CF patients these 9 patients were older and developed pancreatic insufficiency and the presence of *P. aeruginosa* in sputum less frequently. In this group of 9 patients sweat test average values were borderline. Our communication provides data about a single patient and we therefore consider this report of complementary relevance with the worldwide database, particularly for the diagnostic process when a single case is investigated.

In previous works, doubts arising from detection of a rare mutation with uncertain consequences on CFTR function [11] on *cis* with the polymorphic allele IVS8-(TG)12T5 [12] have been overcome by evaluation of NPD functional testing. With the objective of arriving at a conclusive diagnosis in this case, we carried out NPD measurement testing as previously described [13]. Three reliable measurements for the patient were consistent with a CF phenotype. Fig. 1 shows one representative tracing: the Wilschanski index [14] was calculated as equal to 1.23 (non CF values are <0.8 in our Center). Baseline NPD value was -21 mV; the responses to amiloride were 13 mV, 2.8 mV following 0 Cl⁻ and isoproterenol.

We also wondered whether a newly described approach that utilizes peripheral blood monocytes and reports outcomes as an index named “CF index” [15] and where subjects with value scores below 0 are considered to be possible CF subjects, could support the diagnosis in this case. For this patient we calculated a CF index of -18, in keeping with the established diagnosis of CF (Fig. 2). A healthy carrier (F508del mutation) with CF index +22 is shown as reference.

It is worth underlining the fact that this report does not demonstrate the ability of the S977F mutation to cause a CF phenotype on its own but rather can be considered a mutation of varying clinical consequence: subjects need to be homozygous for this mutation in the absence of the T5TG12 genotype to properly assess the disease-causing potential of this mutation. Furthermore, where genetic variants arise, the complete scanning of the gene is required since synergies in different variants/polymorphisms can affect the CFTR function and lead to manifestation of the disease, as in the case of R117H [16].

The diagnosis of controversial cases is challenging and requires the use of sophisticated diagnostic tools [6–9,11,12,16] and for this patient the diagnostic algorithm proposed for CF [3] has been successfully applied. Reporting clinical and electrophysiological information about rare mutations is important for the CF community because it helps to improve diagnostic procedures and to develop better approaches to genetic counseling. Indeed the extensive sequencing being carried out, although essential if diagnostic procedure is to improve, has identified a large number of genetic variants of uncertain functional significance thus limiting the practical application of this valuable information. In these non-classical cases it is critical

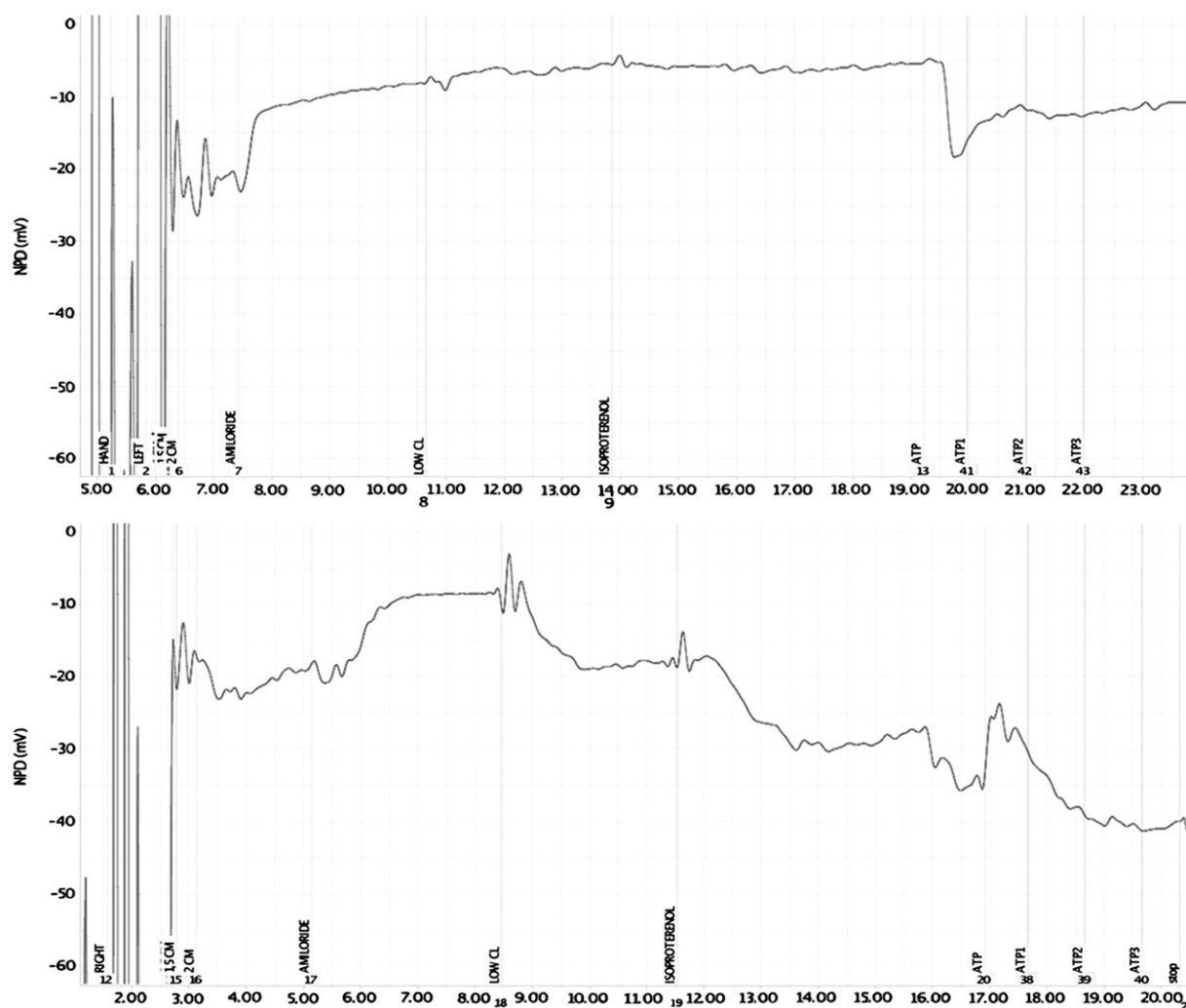


Fig. 1. NPD measurements. The agents indicated at the bottom were added at the times (minutes) shown on the x axis. On the y axis PD are expressed in mV. Top: non-CF subject; bottom: patient. The graph was obtained during measurements in the left nostril and is representative of both nostrils.

to evaluate the functional relevance of rare genetic variants and of complex alleles.

Research in this field is active [17] but validation is necessary and is available only for NPD and ICM [18,19], both of them representing rather invasive approaches. Although no standardized technique is available we are currently testing the value of leukocytes as a source of primary cells useful to evaluate CFTR function. We have recently measured CFTR agonist-dependent membrane depolarization in non-epithelial primary cells and in peripheral blood monocytes [15] as a potential complementary approach to the study of CFTR function. In the case described here we confirmed a correlation between NPD tracings and cell depolarization in monocytes. The role of CFTR in monocytes is currently under investigation and it is hoped that the results will bring new insights to the pathogenesis of CF and sanction a rationale for proposing the use of leukocytes as a potential support for CF diagnosis.

The combined application of DHPLC and NPD analysis in the algorithm for CF diagnosis appears useful for the management of

similar cases. In addition, the development and validation of novel functional assays are expected to improve our diagnostic capability and lead to improved counseling and better treatment of these challenging clinical cases, including the use of CFTR-targeted treatments now in sight.

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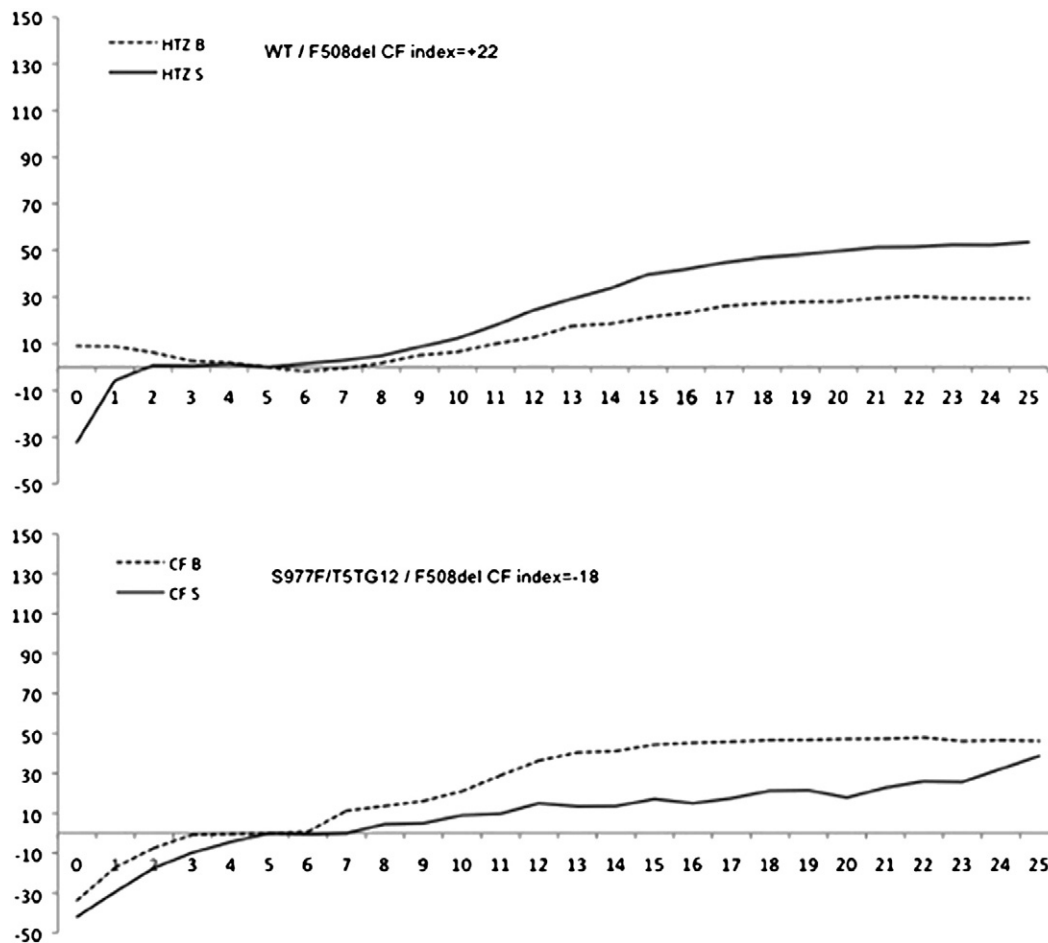


Fig. 2. Monocyte depolarization assay. Single cell fluorescence analysis was performed using DiSBAC2(3) as probe as previously described [15]. Measurements in monocytes were performed in basal conditions (B) and in the presence of stimulus (S) added after 5 min (arrow). Exogenous induction of depolarization was utilized as an internal positive control in monocytes at the end of measurement and in the presence of stimulus (data not shown). The CF index for a healthy carrier (F508del mutation) was calculated to be +22 (top) while the CF index of monocytes derived from the patient was -18 (bottom).

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