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CASE REPORT

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Non pathological sweat test, pancreatic insufficiency and Cystic Fibrosis: an unusual case in a child with F508del-duplication of exons 1–3 CFTR genotype

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Abstract

While Cystic Fibrosis is characterized by a high phenotypic variability, a correlation is reported between the pancreatic status and the *CFTR* genotype. Here we report an unusual case of a child with Cystic Fibrosis (F508del-duplication of exons 1–3 genotype) diagnosed at 8 years old for pancreatic insufficiency and non-pathological sweat test, in absence of respiratory symptoms and acute episodes of pancreatitis. Nasal potential differences and intestinal current measurements were normal, while the short-circuit current measured on patient-derived colonoids grown on Transwell® indicated the presence of a reduced CFTR-dependent current relative to non-CF colonoids with, a modest improvement of CFTR activity record following treatment with elexacaftor/tezacaftor/ivacaftor.

This case opens the discussion on the importance of performing *CFTR* sequencing and the search for large gene rearrangements in cases of pancreatic insufficiency of unclear etiology, also in the presence of non-pathological sweat test. Children with CF and non-pathological sweat chloride are likely to develop higher concentrations if they truly have CF.

Keywords Normal, Sweat chloride, Duplication, Diagnosis, CFTR, Genotype

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Background

Cystic Fibrosis (CF) is an autosomal recessive disorder caused by variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. The CF phenotype is characterized by lung disease (bronchiectasis with persistent airway-based infection and inflammation), exocrine pancreatic insufficiency, associated with nutrient malabsorption, impaired growth, hepatobiliary manifestations, and male infertility [1, 2]. The diagnosis of CF is based on positive newborn screening (NBS), clinical features consistent with CF or a positive family history, in presence of a pathological sweat chloride (SC) concentration (≥60 mmol/L) and/or two *CFTR*-causing variants *in trans* (that is, on two distinct alleles) [3]. More than 2,000 *CFTR* variants have been recorded so far worldwide (http://www.genet.sickkids.on.ca/app, accessed on 19 August 2024) although to date only 719 *CFTR* variants are known to be CF-causing (https://cftr2.org/, accessed on 19 August 2024) based on functional studies. They are typically categorized into six classes, according to their impact on the production, trafficking, functioning or stability of the CFTR channel [4]. A clear and unequivocal genotype–phenotype correlation exists for only a few variants. Also, the variable penetrance of some variants must be also considered. While CF is a monogenic disease, it is characterized by a high phenotypic variability, also in siblings and twins, due to modifier genes other than *CFTR* or to environmental factors [5–8]. A correlation is reported between the pancreatic status and the *CFTR* genotype, given that variants belonging to classes I, II and III usually result in little to no CFTR activity, leading to potentially severe clinical outcomes, pathological sweat test (ST) and pancreatic insufficiency, whilst variants from classes IV, V and VI allow significant residual CFTR function leading to milder phenotypes, sometimes ST in the intermediate range (SC 30–59 mmol/l) and typically pancreatic sufficiency [5–9].

Here we present an unusual case of a child with CF diagnosed for pancreatic insufficiency in presence of a non-pathological ST.

Case presentation

This is the case of an Indian child, not screened at birth for CF, who presented to the local Hospital at the age of 7 years, with gastrointestinal symptoms (chronic diarrhea, recurrent abdominal pain, abdominal bloating) and weight loss (weight < 3rd percentile, -2 SD). The symptoms persisted for 3–4 months in the absence of previous episodes of acute pancreatitis or respiratory symptoms.

Probiotics and proton pump inhibitors were prescribed, as well as different types of dietary regimens (e.g. gluten-free diet, dairy-free diet, no processed food diet), with no improvement of symptoms. Several investigations were carried out to rule out a gastrointestinal pathology: blood exams (including inflammatory markers, ASCA and ANCA serology, and screening for celiac disease), bowel ultrasound scan, microbiologic stool tests and level of fecal calprotectin, with all of them being in normal range. Endoscopic examinations (esophagogastroduodenoscopy and sigmoidorectoscopy) revealed only a minimal focal gastritis associated with *H. pylori* detection.

The patient and his family moved to Italy, where the child was firstly evaluated at our Gastroenterology and Nutrition Unit. Fecal elastase levels were tested, resulting to be below normal range (34 µg/g and <15 µg/g in two different measurements), indicating severe pancreatic insufficiency (for values < 100 µg/g). Consequently, pancreatic enzyme replacement therapy (PERT) was prescribed, improving symptoms and nutritional status (supplemental figure A). To identify the cause of pancreatic insufficiency we performed a ST resulting in the normal range (SC: 26 mmol/L). A chest X-ray was performed in the suspect of Shwachman-Diamond syndrome, excluding the presence of rib abnormalities (Table 1).

Despite non pathological SC value, given the presence of pancreatic insufficiency, *CFTR* gene sequencing with multiplex ligation-dependent probe amplification was carried out. It revealed two *CFTR* variants: F508del and duplication of exons 1–3. Subsequent parental analysis confirmed the presence of most frequent CF causing variant F508del on one allele (inherited from the father) and duplication of exons 1–3 on the other allele (inherited from the mother), not reported in the CFTR2

Table 1 Outcome measures before and after 3 and 12 months of ETI therapy

Patient	SC (mmol/L)	BMI	FEV_1%	NPD	OBAS	ICM (µA/cm2)
Male, 9 y*						
F508del/ duplication of exons 1–3						
Pre ETI therapy	26;38;51	14.08	87	0.39 ± 0.08	0.063	424 ± 261
Three months post ETI therapy	10	15.15	90	NA	NA	NA
Twelve months post ETI therapy	16	14.38	94	NA	NA	NA

* in presence of pancreatic insufficiency and diffuse bronchiectasis at chest computer tomography scan

Abbreviations SC: sweat chloride; BMI: body mass index; FEV_1: forced expiratory volume in 1 second; OBAS Test: Optical Beta-Adrenergic Sweat Test; NPD: nasal potential difference; ICM: intestinal current measurements

database (https://cftr2.org/). Subsequent STs resulted in the intermediate range (SC: 38 and 51 mmol/L). However, next generation sequencing analysis for Shwachman-Diamond syndrome, congenital diarrhea syndromes [10] and genes associated with pancreatitis [11, 12] was negative, excluding the concomitant presence of variants that could explain CF's predominantly gastrointestinal involvement. Given the peculiar presentation of this case we performed additional functional tests, such as Optical Beta-Adrenergic Sweat Test (OBAS Test) [13, 14], nasal potential difference (NPD) measurements [15] and intestinal current measurements (ICM), according to the standardized operating procedure from European CF Society (ECFS) (https://www.ecfs.eu/ctn/standardization-committees) and consensus guidelines from the Cystic Fibrosis Foundation [3]. OBAS test provided a ratio of CFTR dependent sweat rate vs. CFTR- independent sweat rate of 0,063 that is in the range of CFTR related disorders [13] (Fig. 1), while following NPD measurements we calculated a Wilschanski Index (WI) of 0,39±0,08. That is in non-CF range [15]. Again, ICM result was in the normal range (424±261 μA/cm^2) [16] (supplemental figure B).

Pulmonary investigations were performed, revealing diffuse bronchiectasis of grade 1 on the chest computed tomography scan, normal percent predicted forced expiratory volume in 1 s (ppFEV$_1$: 95%) and lung clearance index (6.46, software version 3.3.1, upper limit of normal 7.10) [17]. The patient's culture pharyngeal swab was negative for CF-related pathogens.

Finally, we set to evaluate a potential in vivo benefit of elexacaftor/tezacaftor/ivacaftor (ETI) treatment, in Ussing chamber, using dissociated organoids, derived from the rectal biopsies, cultured in Transwell®, as previously described [18, 19]. Interestingly, non-corrected organoid-derived monolayers (colonoids) responded to the CFTR agonist forskolin with a ΔIsc value of 27±29 μA/cm^2, a result that confirm the presence of residual function and highlight a lower activity than the average recorded for non-CF organoids (ΔIsc 111±15 μA/cm^2), corresponding to a 24% of WT activity in our setting. The ETI treatment led to a modest increment in CFTR activity with Fsk-stimulated currents of ΔIsc 43±29 μA/cm^2 (p=0.7686) reaching approximately 39% of the WT activity in our setting (Fig. 2).

Based on available data a diagnosis of CF was made. In accordance with Italian legislative guidelines, as a child older than six years and with at least one F508del variant, he was eligible for prescription of ETI. The drug affected his SC (10 mmol/l) but not the already normal lung function, assessed under stable conditions three months after the initiation of treatment. Currently, after 12 months of ETI therapy, fecal elastase is still<15 μg/g, with a nutritional status improved dramatically (supplemental figure A).

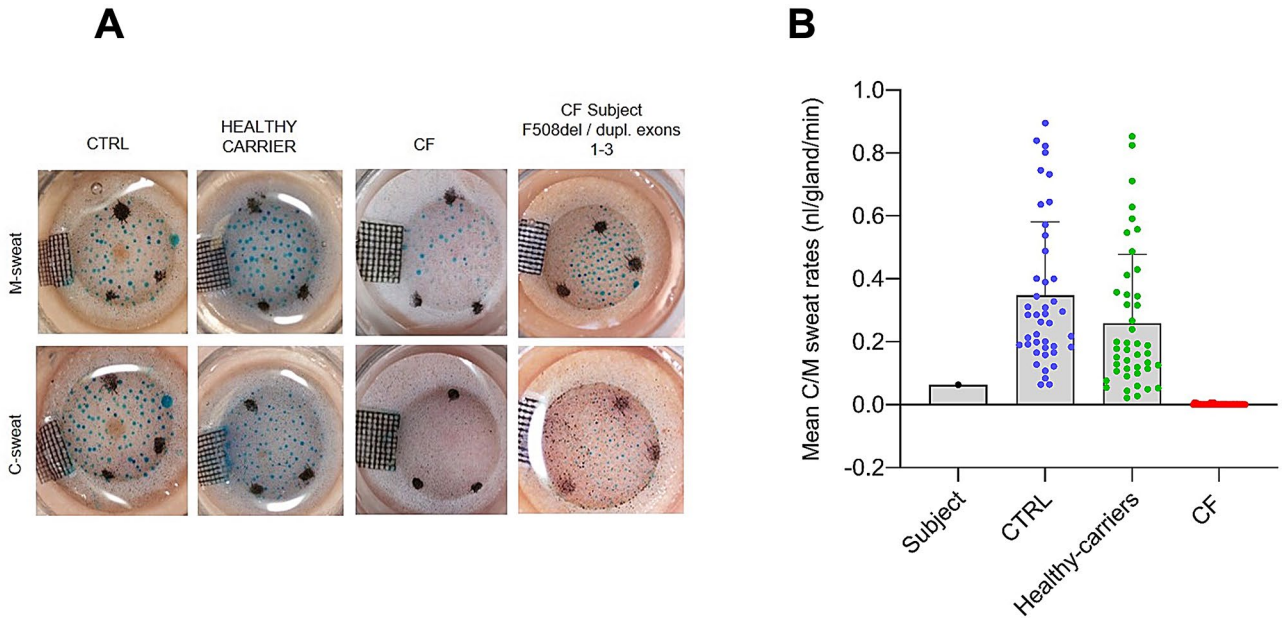

Fig. 1 Representative image of CFTR-dependent and -independent sweat secretion rates for the CF subject under evaluation. (**A**) Each image focuses on a small region of stimulated forearm skin of the selected subjects to show blue-stained sweat droplets by single sweat glands that responded to methacholine (M-sweating) and to the β-adrenergic cocktail (C-sweating), demonstrating the activity of the CFTR channel (C-sweat); grid scale = 0.5 mm. (**B**) Comparison of average C/M sweat rates measured in each group (control: non-CF, healthy carriers: HC, and cystic fibrosis: CF) and patient analyzed (subject)

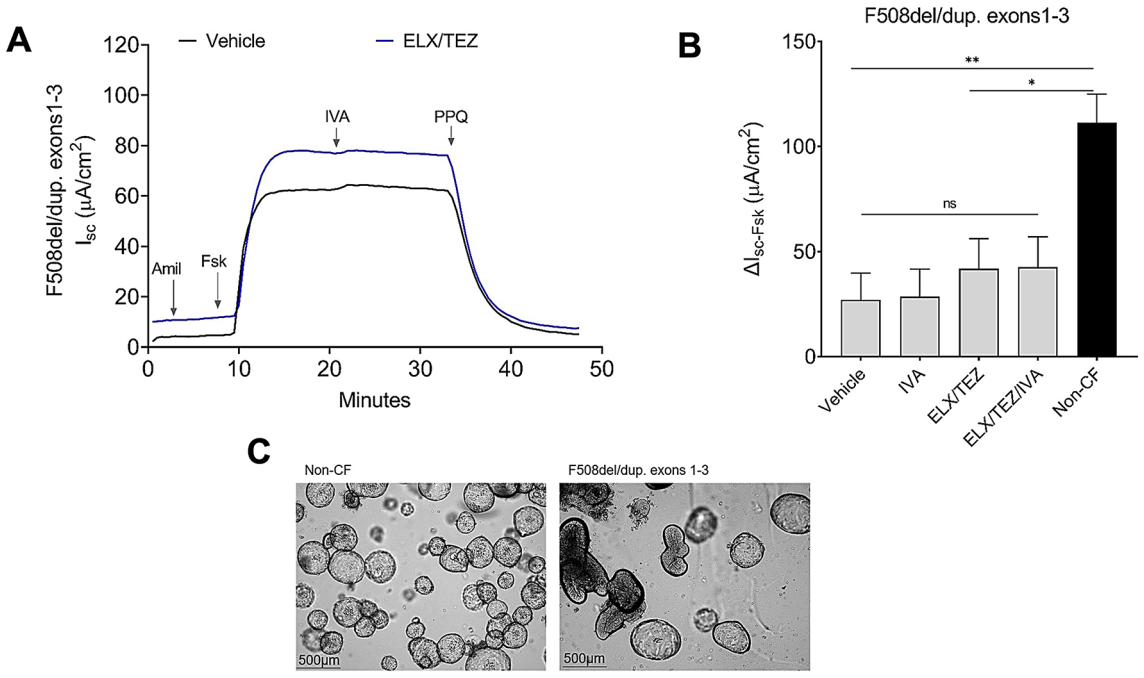

Fig. 2 Functional evaluation of modulator treatment on F508del/dup. exons 1–3 rectal organoids. (**A**) Representative Isc traces of the effect of the vehicle alone (DMSO; black trace), or the correctors ELEXA/TEXA; (ET, 3μM each; blue trace). During the recordings, the colonoids were sequentially treated (as indicated by downward arrows) with amiloride (10 μM), forskolin (10 μM), ivacaftor (0.3 μM), and the CFTR inhibitor PPQ-102 (20 μM). (**B**) Column bar graphic showing mean ± SEMm ($n = 4$). Data reported are the maximal amplitude of the current after addition of forskolin in colonoids pre-treated with vehicle (Veh.) or CFTR modulators. Non-CF mean of ΔIsc values from a minimum of three independent experiments obtained from five non-CF subjects (black column bar) are shown here as reference. Ordinary one-way ANOVA, $*p > 0.05$, $**p > 0.005$. (**C**) Light microscopy analysis of the morphology of Non-CF (used here as reference) and F508del/dup.exons 1–3 intestinal organoids in culture at steady-state condition

Discussion

About 85% of the CF population is pancreatic insufficient early in life, with substantial injury to the pancreas that begins already in utero and correlates closely with the specific *CFTR* variants [20]. However, the extent of injury is variable. Children with CF and pancreatic sufficiency do not have completely normal pancreatic function [21]. It may deteriorate over time, with or without the complicating effects of pancreatitis. Exocrine pancreatic insufficiency may develop without symptoms or may be characterized by failure to thrive in the infant and child or unexplained weight loss in the adult [20, 21].

In the case we observed the child was diagnosed with CF at the age of 8 years, presenting with pancreatic insufficiency and with a non-pathological sweat chloride. However, given the child's age, we cannot exclude future greater lung involvement. As expected in patients with at least one F508del, ETI normalized sweat chloride level and had a positive impact on weight.

This case opens the discussion on the importance of performing *CFTR* sequencing and the search for large gene rearrangements in cases of pancreatic insufficiency of unclear etiology, considering that such variants account for about 6% CF alleles, particularly in ethnic groups with a low frequency of the F508del variant [22].

Furthermore, repeating the sweat test should be considered in the presence of pancreatic insufficiency and first sweat chloride in the normal range. Delayed diagnosis of CF has dramatic consequences on growth and lung function, even more so in children not screened for CF at birth [2].

While the sweat test is one of the main diagnostic tools used in newborn screening programs and one of the main biomarkers of efficacy, in terms of improvement of CFTR protein expression/function by modifying drugs [23–25], it can fluctuate in different diagnostic ranges over time, making a definitive diagnosis difficult in the absence of CF-related symptoms [25, 26]. Furthermore, false positives or negative results have been reported in patients with different diseases or dehydrated or taking drugs that modify chloride levels [27], factors excluded in the case we describe. In addition, there have been reports of children and adults with CF and pancreatic sufficiency, for example, with at least one 3849 + 10KbC > T *CFTR* variant [28–30], who have sweat chloride levels in the normal range (less than 30 mmol/L), although following them and repeating their sweat tests is likely to reveal higher concentrations if they truly have CF.

This is the only case from a retrospective re-evaluation of the 399 patients with CF followed in the regional center of Florence: no other patient had pancreatic

insufficiency at diagnosis with a non-pathological sweat test. Only three adults with pancreatic sufficiency at diagnosis, two *CFTR*-causing variants, and sweat tests in the intermediate range developed pancreatic insufficiency during follow-up, requiring PERT after repeated episodes of acute recurrent pancreatitis.

A similar case with 4016insT/DupPr-ex3 *CFTR* genotype was reported by Paracchini et al. in a child of 4 years with a diagnosis of CF for pancreatic insufficiency and sweat test in the intermediate range (40–41 mmol/l) [31]. DupPr-ex3 is a duplication involving the promoter region (promoter-exon3), also reported in a Chinese carrier with congenital bilateral absence of vas deferens [32].

In the CF mutation database (www.genet. sickk ids. on.ca/) CFTRdup1-3 is described in two unrelated Spanish patients with CF: a male identified by newborn screening, with F508del variant on the other allele and positive sweat test, pancreatic insufficiency and lung disease. The second patient is a male diagnosed at few months with similar clinical features [33].

According to the consensus guidelines from the Cystic Fibrosis Foundation [3], we set out NPD and ICM. Given the peculiar nature of the case we also applied recently proposed but well-established experimental assays such as those based on optical quantification of sweat droplets (OBAS) and analysis of the response of intestinal organoids. OBAS test is performed in vivo and is based on the original study of Wine [34] while application of intestinal organoids in CF has been previously discussed [35]. Interestingly, while the outcomes of the in vivo and ex vivo tests were consistently non-pathological, the sensitive Isc analysis performed in vitro in intestinal organoids detected a functional defect when compared to non-CF controls. The lack of other cell types present in the tissue but not on the colonoids grown as monolayers in Transwell® may play a role in revealing this quantitative difference between assays. Of note, good correlation between the prediction of the efficacy of modulator therapy in vitro using intestinal organoid-based assays and clinical outcomes has been reported in many studies [36].

Conclusions

CFTR sequencing and the search for large gene rearrangements are indicated in case of pancreatic insufficiency of unclear etiology. Initial sweat chloride value in the normal range can be observed in children with two pathogenic *CFTR* variants, but repeated sweat tests might demonstrate higher levels.

The extended analysis of CFTR function was negative except for a lower functional response when CFTR-dependent currents were analyzed in Ussing chambers in epithelial cell monolayers derived from rectal organoids and in OBAS test. Both measurements demonstrated a residual, but significant, CFTR activity consistent with the non-pathological values measured by NPD and ICM. The trend (that does not reach the threshold of statistical significance) to a further increase in CFTR-dependent current following treatment with ETI in the rectal organoids monolayers might suggest a potential benefit of the administration of this drug and is in line with the decreased sweat chloride concentration after ETI treatment. Whether the treatment is active on CFTR protein stabilization (corrector effect, Tezacaftor and Elexacaftor) or probability of channel opening (potentiator activity, provided by Ivacaftor and Elexacaftor) for the individual anions considered (chloride and bicarbonate) is still under evaluation.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12887-024-05154-7.

Supplementary Material 1: Supplemental figure A BMI trend before and after the introduction of pancreatic enzyme replacement therapy.

Supplementary Material 2: Supplemental figure B Recordings of short-circuit currents (Isc). (A) Representative ICM records for anion transport of the CF subject. Experiments were performed in the presence of 10 μM indomethacin. (B) Value for the sum Isc (FI + cch + H) for chloride secretion obtained from the control non-CF group, CF group, and the subject ($n = 4$).

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Author contributions

Conception and design: VT; follow up of the child: VT, CF, AC, SDV, LS, GT, PL; sweat test, microbiological and stool analyses: DD; analysis and interpretation of functional tests: KK, PM, CS; writing the paper: VT. All authors reviewed the manuscript and gave the final approval of the version to be published.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

An approval was obtained from the pediatric ethical committee of the Cystic Fibrosis center in Florence and for the Cystic Fibrosis Center AOUI of Verona (protocol#CFTR050).

Consent for publication

Parents provided written informed consent for anonymous data processing and to participate in this case investigation.

Competing interests

The authors declare no competing interests.

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