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Case Report

The role of improved molecular genetic testing in the diagnosis of cystic fibrosis – a case series

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Abstract

Cystic fibrosis (CF) is diagnosed through a combination of clinical symptoms, a sweat test, and genetic analysis. Over the last five years, significant advances in cystic fibrosis treatment have been driven by a better understanding of the disease's genetic basis. This has enabled the development of more sophisticated genetic tests, allowing for more precise treatment for each individual patient.

We present a case series of children with clinical presentations suggestive of cystic fibrosis, ambiguous sweat test results, and previous "negative" genetic analysis results. More precise and advanced genetic testing revealed that all three patients carried two CF-causing mutations.

In cases where there is high clinical suspicion, despite initial results indicating a negative outcome, we recommend the use of MLPA analysis for the detection of large deletions and insertions that cannot be detected by standard sequencing methods.

Keywords

asthma, chromosomal deletion, failure to thrive, hepatomegaly, sweat test

Introduction

Cystic fibrosis (CF) is an autosomal-recessive inherited disease with an established protocol for diagnosis based on well-known clinical presentation and laboratory confirmation, including sweat tests and genetic analysis.[1] However, with the development of better molecular genetics testing, more cases of unusual or atypical CF patients are reported, and new pathogenic mutations are gradually reclassified from variants of uncertain significance. These improvements are also important in the newborn screening programs and especially in cases with CF-screening-positive inconclusive diagnosis (CF-SPID) or CF-related disorders.[2]

In light of the availability of highly effective modulator therapy in the last five years, the prognosis of the disease improved dramatically, and the results of genetic analysis are the basis of the therapy response.[3] Several ethnic groups have distinct mutation distributions, but common knowledge is country-specific—screening programs include a set of mutations that account for more than 90% of all mutations in the population.[4]

We present a case series of children with clinical presentations suggestive of CF, ambiguous sweat test results, and initial "negative" results from the genetic analysis. During the follow-up process, all patients were genetically retested and confirmed to have two CF-causing mutations.

Case reports

The first case is a 10-year-old boy with a family history of paternal pollinosis. At the age of one month, he was treated in the local hospital for bronchiolitis and diarrhea. During the next two months the child was hospitalized once for pneumonia and the second time for a respiratory tract infection and anemia. From the age of two months, the infant exhibited signs of failure to thrive (FTT). Based on the presence of seborrheic dermatitis and a family history of atopy, the possibility of asthma and a cow's milk protein allergy was considered. At the age of four months, he was referred to a CF clinic, but his sweat test results were borderline, and blood was sent for genetic testing—only one disease-causing mutation was confirmed—c.1521_1523delCTTtr (p.Phe508del). Since the patient was of Roma ethnic origin, and it was postulated that 100% of these patients in the country are homozygous for c.1521_1523delCTTtr, he was diagnosed as a healthy carrier. All clinical symptoms were explained with the poor social status of the family and the false-positive borderline sweat test. During the following years, the boy had four episodes of wheezing and was treated as an asthmatic patient. Due to a prolonged "asthma attack" of 3 weeks, he was eventually referred to the CF clinic, and the performed tests showed a borderline sweat test (50 mEq/l), cylindrical bronchiectasis on the computed tomography scan, sputum positive for Pseudomonas aeruginosa, and a negative screening test for celiac disease. Weight and height were in the 26th and 53rd percentiles, respectively, with a BMI of 15.4 (z-score - 0.98, 16th percentile). A new blood sample was sent for molecular genetics analysis, and in addition to one c.1521_1523delCTTtr, another mutation was confirmed—a deletion of exon 2 (CFTRdele2). After ten years of wrong diagnosis and therapy, the initial suspicion for CF was confirmed, and a complex management plan was prescribed.

The second case involves a 7-year-old boy of Roma ethnic origin. At birth, the child was operated on for meconium ileus and had a complicated post-surgical recovery with confirmed infections with MRSA, Pseudomonas aeruginosa, and pancreatic steatorrhea. Despite two positive sweat tests—61 mEq/l and 63 mEq/l—the diagnosis of CF was dismissed due to genetic analysis that showed no c.1521_1523delCTTtr (p.Phe508del) mutation. Seven months later, due to severe tiredness and electrolyte disturbances (hyponatremia, hypochloremia, and hypokalemia), the patient was admitted to a CF specialized unit, where a repeat molecular genetics analysis was ordered, which confirmed the compound heterozygous c.1393-1G>A and c.3209G>A (p.Arg1070Gln)-c.1397C>G (p.Ser466Ter) mutations. A complex treatment for CF was initiated, and the child currently is free of MRSA and Pseudomonas aeruginosa.

The third case is an 11-year-old girl who initially presented at the age of two months with failure to thrive. She had a sweat test performed at the age of five months with a negative result of 20 mEq/l and a negative molecular genetics analysis for CF for the known mutations for the population in the country. She has since complained of abdominal bloating and paraumbilical pain, as well as thicker stools at times. Since the age of seven years, she has had frequent respiratory tract infections due to sinusitis, always treated with antibiotics for at least 10 days and corticosteroids. Eventually, at the age of eleven years, she underwent a liver ultrasound, and chronic liver disease similar to CF-related liver disease was reported. Thus the patient was referred to the CF clinic. Notable findings included short stature, severely reduced subcutaneous fat mass, weight in the second centile and height in the fourth centile with a BMI of 15.4 (z-score - 1.15, 12th centile), enlarged abdomen with hepatomegaly, varicose bronchiectasis on CT scan, sputum positive for P. aeruginosa, a negative scan for celiac disease, and two positive sweat tests—64 and 65 mEq/l. A new blood sample was sent for molecular genetic analysis, and two disease-causing mutations were confirmed: c.3731G>A (p.Gly1244Glu) and deletion of exons 2 and 3. Similarly to the first case, it took more than ten years to confirm the initially suspected CF. Summarized comparable data for the three cases are shown in ***Table 1****.*

Discussion

Since the discovery of the sweat test and the CFTR gene, there have been well-established criteria for an early diagnosis.[5] Before introducing the newborn screening (NBS) programs, the diagnosis was mainly based on the clinical presentation, and the mean age of diagnosis was higher than 3.6 months, as currently reported by the European CF patient registry.[5,6] In 2024, many countries have programs for screening, and they diagnose most of the children within the first two months of life; in other countries, however, the diagnosis is delayed. NBS results in an earlier diagnosis, earlier treatment, and better health outcomes. However, it also can lead to missed cases and uncertain cases that need to be followed up.[7] Most likely, even if we had NBS in our country, all three cases would have been classified as screening-negative or at most as CF-SPID for several reasons.

The first case probably would pass as a positive on immunoreactive trypsinogen (IRT) level due to carriage of *c.1521_1523delCTTtr (p.Phe508del)* and confirmed later on the second mutation; however, he would fail on the sweat test level and on the genetic analysis.

The most common CF mutation is *c.1521_1523delCTTtr (p.Phe508del)*, with over 91600 confirmed cases worldwide. [8] Prior to 2019, it was thought that all Roma patients in our country were 100% homozygous for this mutation. However, more recent data have shown different results. [4,9] The patient was born before 2019, and even with positive IRT (if performed) and only one *c.1521_1523delCTTtr (p.Phe508del)*, he would not have been considered a CF patient.

Sanger sequencing and NGS sequencing are not suitable for detection of large deletions and insertions in the CFTR gene. The multiplex ligation-dependent probe amplification (MLPA) analysis is more appropriate. MLPA, however, is not often part of the NBS programs, and with a

Table 1. Detailed patient data at initial assessment and at the time of diagnosis

	Case 1	Case 2	Case 3
Initial presentation	Recurrent respiratory tract infections and FTT	Meconium ileus	FTT
Initial sweat test(s)	50	61	20
Initial molecular genetic analysis	One F508del	No mutations typical of Roma CF patients in the country were found	Overruled 95% of mutations for the patient population in the country
Time of the next evaluation	10 years	7 months	10 years
Presentation at diagnosis	Prolonged asthma attack	Electrolyte disturbances	Suspected CF-related liver disease
Second sweat test	50	63	62
Final molecular genetic analysis	c.1521_1523delCTTtr / CFTRdele2	c.1393-1G>A and c.3209G>A (p.Arg1070Gln), c.1397C>G (p.Ser466Ter)	c.3731G>A / CFTRdele2, 3
Physical examination and laboratory findings	PA positive bronchiectasis	PA and MRSA positive Hypokalemia	PA positive bronchiectasis Hepatomegaly
Therapy	ERT, inhaled antibiotics, mucolytic	ERT, inhaled antibiotics, nutritional support	ERT, inhaled antibiotics, mucolytic
CFTRm therapy	Eligible, but parents need time to decide for it	Non-eligible	Eligible, on a treatment for one year
Current status	Improved status, no pulmonary exacerbations for the last 6 months	No PA, no MRSA, developing normally	Improved weight and height gain (BMI 16.9), no abdominal symptoms, no pulmonary exacerbations

FTT: failure to thrive; PA: *Pseudomonas aeruginosa*; MRSA: methicillin resistant *Staphylococcus aureus*; ERT: enzyme replacement therapy; CFTRm: cystic fibrosis transmembrane regulator therapy; BMI: body mass index

borderline sweat test, the child would be mostly classified as CF-SPID.[1,7] The deletion of exon 2 (CFTR dele ex2) is a large deletion variant that affects the quantity of CFTR proteins.[10] Currently 148 patients are reported with this variant, and they all have similar data for lung function, pancreatic insufficiency, and *P. aeruginosa* infection levels compared to the general CF population.[11]

Since we have confirmed two mutations (one of them eligible for modulators) in this patient, we offered the parents CFTR modulator therapy, but they are currently reluctant to do so. One possible explanation for their hesitancy is the 10-year period during which they were told the child only had asthma, and they are still working on accepting the diagnosis. Non-acceptance or problems with coping with the diagnosis can lead to treatment non-adherence and thus worsen the health outcomes for the patient.[12]

The second case would definitely also be negative on presumed NBS as he presented with meconium ileus, and a well-known fact is that patients born with meconium ileus have negative screening on first step (which is usually IRT).[13] Additionally the rare genetic mutations were not reported in our country prior to the birth of this baby. The first mutation, c.1393-1G>A, is a variant to the splice donor/acceptor sites that affects CFTR quantity.[14] The variant was described as pathogenic and reported in 105 patients in CFTR2 database, based on which the patients with this mutation are with similar lung function and sweat chloride levels as general CF population, but have higher incidence of *P. aeruginosa* and of pancreatic sufficiency.[15] Two patient reports were published in a heterozygous Pakistani patient with classical CF presentation and in a homozygous Serbian patient with atypical presentation.[16,17] The second mutation is a rare complex allele *c.3209G>A (p.Arg1070Gln)- c.1397C>G (p.Ser466Ter)* involving a nonsense and a missense variant and thus affects the CFTR quantity. It was described in 56 patients with similar lung function, sweat tests, and *P. aeruginosa* and higher pancreatic insufficiency prevalence compared to general CF population.[18]

In the majority of newborn screening programs, infants with meconium ileus are typically referred for molecular genetic analysis. However, in the case of the first child in our country to exhibit such genetic mutations, the likelihood of a missed diagnosis is significantly increased unless the test is repeated with an expanded genetic panel, as was done in our case.

The third case would also be missed for almost the same reasons as aforementioned cases 1 and 2. Even with positive IRT, her first sweat test was negative, and the mutations found were not reported in our country database prior to 2019. The *c.3731G>A (p.Gly1244Glu)* is a missense

variant that affects both CFTR quantity and function; it is also classified as a gating mutation.[19,20] It is confirmed in 186 patients worldwide, and they have lower *P. aeruginosa* infection rates compared to the general population.[21] It is one of the most common mutations in Basilicata, Italy, where it is reported in about 9% of their CF patients.[22] The gating class mutations were the first to have an approved CFTR-modulator therapy[23], so we offered the treatment to the patients when the second genetic analysis confirmed *c.3731G>A (p.Gly1244Glu)*. The modulator therapy was initiated even before the discovery of the second mutation, but we have confirmed the diagnosis clinically, and the repeated sweat test was positive.

The second mutation, which was also confirmed through MLPA, is a large deletion variant that affects CFTR quantity. It is confirmed in 1270 patients worldwide and they show higher levels of pancreatic insufficiency and lower in *P. aeruginosa* infection, compared to the general CF population.[24] It is a common finding in patients with Slavic origin.[25]

All three patients have had their final diagnoses confirmed, and they are currently receiving appropriate basic CF care. Only one of the two eligible patients has agreed to receive modulator therapy, and she has shown the greatest improvement compared to the other two.

Conclusion

Molecular genetics is an intriguing tool not only for clinical purposes but also for genealogy research. In the presented cases, it took more than ten years to confirm the initially suspected disease, resulting in complications such as chronic *P. aeruginosa* infection and bronchiectasis. Deleted alleles account for approximately 1% of molecular defects in our population, so they are not that uncommon. In cases with high clinical suspicion, despite initial "negative" results, we recommend MLPA analysis for detecting large deletions and insertions that cannot be detected using standard sequencing methods.

Ethics clearance

Written informed consent to publish patient information was provided by the parents of the patients.

Conflict of interest

The authors have no conflicts of interest to declare.

Financial disclosure

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