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Short Communication

[R74W;R1070W;D1270N]: A new complex allele responsible for cystic fibrosis

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Abstract

Since the beginning of population screening for CF carriers, it has become apparent that complex *CFTR* alleles are not uncommon. Deciphering their impact in disease pathogenesis remains a challenge for both clinicians and researchers. We report the observation of a new complex allele p.[R74W+R1070W+D1270N] found in *trans* with a type 1 mutation and associated with clinical diagnosis of cystic fibrosis in a one year-old Moroccan patient. This case underlines the difficulties in counseling patients with uncommon mutations and the necessity of functional studies to evaluate the structure–function relationships, since the association of several variations in *cis* can dramatically alter CFTR function.

Keywords: Complex allele; R74W; D1270N; R1070W; CFTR; Cystic fibrosis

1. Introduction

More than 1700 cystic fibrosis transmembrane conductance regulator gene (*CFTR*) mutations and polymorphisms are currently reported in the *CFTR* mutation database [1]. Although commercial kits are available to identify the most frequent cystic fibrosis (CF) mutations, most known mutations appear at frequencies below 0.1% or represent private mutations. In addition, a significant percentage of CF mutant alleles (ranging from 2 to 10%) still remains unidentified in CF patients [2]. Since the beginning of population screening for CF carriers, it has become apparent that complex alleles such as [R74W; D1270N] (HGVS nomenclature: c.[220C>T;3808G>A]) are not uncommon [3,4]. Complex alleles are thought to affect the expression of the phenotype by modulating the effect of mutations [5–9] but further data are needed to clarify their functional role. We report the observation of a new complex allele [R74W;R1070W;D1270N] associated with cystic fibrosis in a patient from a Moroccan family.

2. Case report

The patient is the first female child of a Moroccan non-consanguineous couple. Family history is unremarkable and notably there is no known CF case. She was born at term after an uneventful pregnancy and weighed 3780 g (P75–P90) for 48 cm height (P3–P10). She presented feeding difficulties and vomiting since birth. She was hospitalized in Morocco at the age of 3 months for dehydration, signs of malnutrition and diarrhoea. After rehydration, biological analyses revealed hyponatremia, hypochloremia and hypercalcemia with elevated natriuria. Back in Switzerland, she was hospitalized for gastroenteritis and malnutrition. Cow's milk protein intolerance was ruled out and cystic fibrosis was suspected. Sweat conductivity measurements (Nanoduct Neonatal Sweat Analysis

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System, Wescor) showed high chloride values at 85 mmol/L (sweat production 3.3 g/m^2/min) and 89 mmol/L (sweat production 6.7 g/m^2/min). At that time the sweat chloride test did not allow to collect sufficient amount of sweat. A treatment with pancreatic enzymes (Creon) was started to support weight gain although she presented no pancreatic insufficiency (pancreatic elastase values at 360–380 mcg/g and normal ultrasonography). Metabolic alkalosis was treated by NaCl administration (4 mEq/kg/j). One month later, the patient was rehospitalized for a clinical and radiological picture of pneumonia. Throat swab culture remained negative.

The conventional sweat chloride test was repeated at the age of 9 months, allowing to collect 54 μL of sweat: chloride value was 44 mmol/L, in the intermediate range. Sodium value was 40 mmol/L and the sweat Na:Cl ratio was 0.91. She is followed by the specialized cystic fibrosis consultation and benefits from physiotherapy. During follow-up, several episodes of rhinorrhea were reported and throat swab culture showed the presence of Hemophilus influenzae. Creon has been stopped when she was one year-old. Pancreatic elastase is measured every three months and remains in the normal range. At 13 months her growth curve follows P10–P25 for weight and P50–P75 for height. The patient's evolution under medical supervision is compatible with symptomatic CF.

Molecular analysis using a commercial kit (INNO-LiPA, Innogenetics, Ghent, Belgium) identified the heterozygous type I splice-site mutation 711+1G>T (HGVS nomenclature: c.579-1G>T). Analysis of the entire coding sequence of the gene was performed in order to find the presumed second mutation. This search confirmed the previous result and found three further *CFTR* variants in heterozygosity. Segregation analysis showed the father to be a carrier of 711+1G>T, and the mother of a complex allele [R74W;R1070W;D1270N] (HGVS: c.[220C>T; 3208C>T; 3808G>A]), confirming our patient as a compound heterozygote.

3. Discussion

Here we report a new complex allele [R74W;R1070W; D1270N] in *trans* with a type I *CFTR* mutation in a patient with clinical diagnosis of CF and elevated sweat conductivity measurements. Nevertheless, sweat chloride test is in the equivoqual range and molecular analysis identified a new CF complex allele of unknown significance.

The sweat chloride test remains the gold standard for CF diagnosis but does not always give a clear answer [10]. Guidelines from the Cystic Fibrosis Foundation Consensus Report established that sweat chloride values ≥40 mmol/L in individuals over age 6 months should be considered beyond the normal range and merit further evaluation [10].

In our case, although sweat test did not allow to confirm diagnosis, the Na:Cl ratio <1 is supportive of a diagnosis of CF in children [11]. Moreover, a recent Swiss study comparing conductivity sweat test versus chloride concentration test concluded that conductivity test measurement >80 mmol/L allowed CF diagnosis with a good sensibility and sensitivity [12].

Since CF is associated with a wide range of disease states, it is extremely difficult to predict the phenotype for newly identified mutations. The association of mild CFTR mutations or variants in *cis* may have a more deleterious effect than each mutation alone [7]. Yet, the effect of most complex alleles remains controversal or unclear in the literature. The double mutant allele [R74W;D1270N], first described in 1995, was originally thought to be deleterious, although considered as a "mild" *CFTR* mutation responsible for a congenital bilateral absence of the vas deferens (CBAVD) phenotype [7,13]. However, it has since been found in asymptomatic individuals and may not be sufficient to cause disease [14]. The same authors described the triple mutant [R74W;V201M;D1270N] associated with CBAVD when found in homozygous state or in *trans* with a severe CF mutation [14]. More recently, another group reported healthy male dizygotic twins carrying [R74W; V201M;D1270N] in *trans* with F508del, suggesting that this complex allele is not associated with classical CF [15]. To our knowledge, R1070W has never been described within a complex allele. R1070W is considered a mutation of "mild" pancreatic-sufficient CF or of CFTR-related disease including CBAVD [1,16]; functional studies have revealed abnormal localization of CFTR bearing R1070W [16].

It is difficult to evaluate the contribution of each mutation to the phenotypic expression. Prediction tools such as SIFT, POLYPHEN fail to account for missense mutations that are found as complex alleles. In a triple mutant, the variations in *cis* can act in concert to alter CFTR function dramatically. Nevertheless, functional studies will be needed to evaluate the structure–function relationships.

The patient's phenotype suggests that this novel complex allele might significantly impair the function of CFTR. Given the young age of the patient, pancreatic insufficiency in later life cannot be ruled out. Recently, the prevalence of CF and CF-related disorders in the Moroccan population was estimated to be in the range of that found in European populations [17]. Family information and carrier testing within relatives were therefore recommended and the availability of prenatal diagnosis during a future pregnancy of the parents or other at-risk relatives was explained.

Research on new treatments for CF now focuses on developing new pharmacological approaches to address specific CFTR dysfunctions, further underlining the importance of complete *CFTR* sequencing and confirmation of the inheritance of mutations from both parents in order to establish proper diagnosis. Complex alleles and their role in disease pathogenesis still remain a challenge for both researchers and clinicians.

Conflict of interest

The authors declare no conflict of interest.

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