Reports of Three Cases of Atypical Cystic Fibrosis with Azoospermia

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Abstract

Cystic Fibrosis is the most common autosomal recessive genetic disease in Caucasian population. Extending knowledge about the molecular pathology on the one hand allows better delineation of the mutations in the CFTR gene and the other to dramatically increase the predictive power of molecular testing. This study wants to underline that the identification of individuals with atypical cystic fibrosis can sometimes present particular difficulties of interpretation. On that ground, if there is a strong clinical suspicion, it is always advisable the biochemical study by performing the sweat test, followed by sequencing of the CFTR gene. Not all patients with cystic fibrosis have abnormal sweat chloride levels, severe lung disease, or failure to thrive. These three cases remind us to think “outside the box.” For these reasons, in patients with idiopathic azoospermia, it is important to perform the sequencing of CFTR gene.

Abbreviations: CFTR: Cystic Fibrosis Transmembrane Regulator; CF: Cystic Fibrosis; CBAVD: Congenital Bilateral Agenesis Of the Vas Deferens

Introduction

Cystic Fibrosis (CF) is the most common autosomal recessive genetic disease for the Caucasian population. In Italy, the disease occurs in 1/2500 to 1/3000 Caucasian newborns, with a carrier incidence ranging from 1/26 to 1/30 in the general population [1, 2]. CF is a complex multisystem disease related to the buildup of thick, sticky mucus that can damage many of the body’s organs (epithelia of the respiratory tract, exocrine pancreas, intestine, male genital tract, hepatobiliary system, and exocrine sweat glands). The cystic fibrosis transmembrane conductance regulator gene (CFTR) is located on the long (q) arm of chromosome 7 (7q31.2) [3, 4].

More than 1,800 mutations in the CFTR gene have been identified [5]; many of which are so rare as to be called ‘private’ as they are only present within individual families.

Besides the classical form of severe disease, other clinical forms of CF have been identified and called “atypical” forms.

This form of cystic fibrosis may be accompanied by one or more of these alterations: congenital bilateral agenesis of the vas deferens [6], asthma and bronchiectasia [7], recurrent pancreatitis [8].

Recently, the studies on the genetics of CF have followed different directions, showing that there are a number of proteins that interact with the CFTR increasing up to six times the activity, like the SLC26 carrier family [9]. Mutations in these genes may result in a defective activation of CFTR [10]. Not all patients with cystic fibrosis have abnormal sweat chloride levels, severe lung disease, or failure to thrive. These three cases remind us to think “outside the box”.

This paper aims to identify the individuals with atypical cystic fibrosis through the study of the CFTR gene. In presence of clinical suspicion of atypical cystic fibrosis it is useful to perform the sweat test followed by sequencing of the CFTR gene [11].

Case 1

A caucasian couple came to our laboratory to perform tests of karyotype and molecular screening of the CFTR gene, a preliminary examination for the Assisted Reproductive Technology (ART) . The man had shown multiple episodes of chronic sinusitis and had been undergone two operations of nasal polyposis. He had not shown other signs that could raise the suspicion of CF.

Testing

Molecular screening of the CFTR gene (commercial kits produced by Nuclear Laser Medicine S.r.l., Settala (MI), Italy) has shown that the lady don’t have the mutations sought (wild type), while her husband was heterozygous for the mutation G542X. Pancreatic and liver biochemical values were normal. At a later stage he has been undergone urological tests which have shown that the patient presented congenital bilateral agenesis of the vas deferens (CBAVD). Performing a semen analysis, we have noticed that the patient had azoospermia. Analysis of the karyotype of both spouses which has been obtained from T lymphocytes, extracted from peripheral blood, by using the common culture technique. The obtained chromosomes were banded with Q-banding methods using quinacrine and it was normal for both. A sweat chloride test was positive (> 60 mmol/L) on both arms: 99 and 108 mmol/L.

A semen analysis performed on both partners has shown the presence of spermatids and spermatozoa. On the second day of the menstrual cycle the patient had shown multiple episodes of chronic sinusitis and had been undergone two operations of nasal polyposis. He had not shown other signs that could raise the suspicion of CF.

The man then was found to be compound heterozygous (G542X/G542X) for the CFTR gene. Pelvic ultrasonography, performed on a lady has showed the presence of a retroflexed uterus, with a normal profile, echostructure and dimensions. Her endometrium had a normal echographic aspect. Both her right and left ovary were normal in relation to the dimension and form, without any liquid effusion. A hysterosalpingogram confirmed the normal uterine-tubal anatomy. Serum anti-ovarian and anti-adrenal antibodies were absent. On the third day of the menstrual cycle the patient had normal levels of gonadotropins (LH: 6.4 IU/L and FSH: 7.3 IU/L). Her thyroid-stimulating hormone, free tri-iodothyronine and free thyroxin hormone levels were normal, while the levels of anti-thyroid peroxidase antibodies and anti-thyroglobulin antibodies were normal. The lady was not carrying any of the 60 mutations studied by dot blotting reverse. In order to prevent that the woman might be a carrier of a mutation not included in the 60 studied, we also proceeded to a complete CFTR gene sequencing which have confirmed the absence of mutations [12].
Treatment

We have explained to the couple that the possibility to conceive a child or daughter with CF does not exist, but they will be carriers of the G542X mutation or mutation E83I X.

The genetic structure found in the husband, has shown a very good chance to find sperm in the testicles. Consequently the couple may try to become biological parents, undergoing a cycle of in vitro fertilization (IVF).

Case 2

A 29 year old Caucasian man came to our laboratory to perform the spermiogram. The azoospermia was the only clinical sign observed.

Testing

An examination of seminal fluid was observed azoospermia with low seminal fluid volume (0.8 ml). After a urological examination it has been observed the patient presented congenital bilateral agenesis of the vas deferens (CBAVD).

As a consequence, we have performed a molecular screening of the CFTR gene with reverse dot blot (commercial kits produced by Nuclear Laser Medicine S.r.l., Settala (MI), Italy). Molecular analysis of the CFTR gene has shown that the patient was a compound heterozygote for mutations G542X/D1152H.

Molecular screening of the CFTR gene has shown that the wife not had the mutations sought (wild type).

Because of the patient’s genotype we decided to extend the examination to blood relatives, and a brother of 26 years who after molecular screening of the CFTR gene was also compound heterozygous for mutations G542X/D1152H.

Treatment

The genetic structure found in the boy has shown a good chance of finding sperm in the testicles. The patient has undergone testicular biopsy (testicular sperm extraction: TESE).

The genetic structure found in the boy has shown a good chance of finding sperm in the testicles. The patient underwent testicular biopsy. The couple, with sperm collected through testicular biopsy was able to become biological parents.

Case 3

A 37 year old Caucasian male, came to our laboratory to run the screen molecular CFTR gene. The patient suffered from pancreatic insufficiency. When he arrived he reported to have already performed a semen analysis which showed azoospermia.

Testing

Running the sweat test, the result wasn’t exhaustive with values 55 mmol/L [13]. By molecular screening of the CFTR gene with reverse dot blot, the molecular analysis of the CFTR gene was performed following these steps:

- DNA isolation, starting from 25 μl of venous blood collected in EDTA-K3.
- Polymerase chain reaction (PCR) and reverse hybridization.

The test has sensitivity and a specificity of more than 99%, with a direct analysis of 60 mutations of the CFTR gene.

The patient was heterozygous for the F508del mutation. The strong clinical suspicion made us proceed to the sequencing of the 27 exons of the CFTR gene. The sequencing of the gene has shown a second mutation L636P! In literature has been described only one case with the above mutation [14]. The patient showed normal pancreatic function and a sweat test within normal limits, but evidence of bronchiectasis.

Treatment

The patient has resulted heterozygous compound for the mutations F508del/L636P, with allelic variant 7T/9T. The patient does not show particular clinical problems, and can become biological father with a withdrawal of sperms in the testicles if they are present.

Results and Discussion

The aim of this work is to highlight how it is possible to present clinical dubious forms with few clinical indications. Until today we have been identified in more than 1800 mutations in the CFTR gene with different penetrance. The authors want to highlight the importance of clinical signs that can lead to suspect a diagnosis of CF and to consider the possibility of the sequencing gene if there are suspicious clinical examinations with doubts and inconsistent values. The diseases in these patients didn’t present the classic clinical signs, such as lung problems, pancreatitis, malabsorption, high level of chloride in the sweat. In forms with classic signs, the disease was diagnosed during childhood [15]. The cases presented can be defined as unusual and do not show the typical signs of the disease and have been unnoticed in the childhood, but they have been discovered only at an adult stage by showing problems of procreation. In some cases the sweat test was negative or borderline. This work underlines the importance of a molecular test, since it is the only means available today to give a reliable diagnosis.

Consent

Written informed consent was obtained from all of the patients (including legal guardians of the children) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal. Our local institutional ethics committee approved this study.

Competing Interests

The authors have declared that no competing interests exist.

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