

CYSTIC FIBROSIS - CLINICAL AND GENETIC EVALUATION AS AN APPROACH FOR EFFECTIVE PREVENTION

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ABSTRACT

Cystic fibrosis (CF) is a common AR hereditary disease (1:2500 newborn). The contemporary attainments in the field of molecular genetic diagnostics of CF create opportunities for effective prevention of the disease. The clinical and genetic evaluation includes the following stages: 1. Clinical diagnosis - based on typical clinical presentation at different ages (neonates, children, adults). 2. Laboratory confirmation of the clinical diagnosis - sweat test performed twice with results over 60 meq/l Cl-. 3. DNA analysis: a) Identified mutations of CFTR gene or polymorphic markers determine the family as "informative" and this makes prenatal diagnosis in next pregnancies possible - secondary prevention. b) Identifying mutations of CFTR gene in a patient with CF makes possible testing for heterozygote status in close relatives, and when such is found prenatal diagnosis is possible - primary prevention.

This clinical genetic approach for evaluation was applied to 31 patients with CF and their families. 51 mutations were idnetified - 36 (70.5%) ?F508 and 15 (29.5%) other mutations. Prenatal diagnosis as a secondary prevention was performed in 8 pregnancies in informative families and one was terminated, because the fetus was found to be homozygote. Five of the tested close relatives of patients with CF were found heterozygotes; 3 partners of confirmed heterozygotes were tested for evaluating the necessity of performing prenatal diagnosis of CF, but all were found healthy homozygotes.

In conclusion, the collaboration between pediatricians, pulmonologists, gastroenterologists and geneticists will improve the prevention of CF.

Keywords: cystic fibrosis, DNA analysis, prevention

INTRODUCTION

Cystic fibrosis (CF) is a chronic, progressive, and frequently fatal autosomal recessive hereditary disorder of the body's mucus glands. Cystic fibrosis primarily affects the respiratory and digestive systems in children and young adults. The sweat glands and the reproductive system are also usually involved. On the average, individuals with cystic fibrosis have a lifespan of approximately 30-35 years. It affects one out of 2500 live births. However, this frequency varies according to geographic and ethnic origin of the patients (fig.1). Also, about 1 in every 25 is an unaffected carrier of an abnormal "CF gene." When both parents are carriers, the recurrence risk is 25%.

The CF gene was identified in 1989. It is localized on 7q31 and contains 27 exons. The biochemical abnormalities in CF results from a mutation in this gene that produces a protein responsible for the movement through the cell membranes of chloride ions (a component of sodium chloride, or common table salt). The protein is called CFTR-cystic fibrosis transmembrane regulator. Since then, a great deal has been learned about this gene and its protein product and more than 1500 mutations were identified (4,7,8).

The purpose of the present paper is to introduce an effective approach for prevention of CF in the population.

Race or ethnicity	Chance of having a baby with CF
White (European ancestry)	1 in 2 500
Hispanic American	1 in 8 500
African	1 in 17 000
Asian	1 in 32 400

Fig.1 Incidence of Cystic fibrosis according to the ethnic origin

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MATERIAL AND METHODS

The clinical and genetic evaluation of CF patients as an approach for effective prevention of the disease in the population includes the following stages:

I. Clinical diagnosis:
   - In newborns - in 10% of the affected newborns, CF presents as meconium ileus (intestinal obstruction due to abnormally thick meconium) (1).
   - In infants and young children - they should be tested for cystic fibrosis if they have persistent diarrhea, bulky foul-smelling and greasy stools, frequent wheezing or pneumonia, a chronic cough with thick mucus, salty-tasting skin, or poor growth. Usually the symptomatology involves two major organ systems, respiratory and digestive, and the digestive involvement with exocrine pancreatic insufficiency is seen in 85% of the patients, leading to the obstruction of pancreatic ducts and thus lipoprotein malabsorption. The state of exocrine pancreas serves as a marker of the gravity of the phenotype. (8). The result is malnutrition, poor growth, frequent respiratory infections, breathing difficulties, and eventually permanent lung damage. Lung disease is the usual cause of death in most patients.
   - In adults - sometimes signs of the disease may not show up until adolescence or even later. CF can cause various other medical problems. These include sinusitis, nasal polyps, clubbing, pneumothorax, hemoptysis, cor pulmonale, abdominal pain and discomfort, gassiness, and rectal prolapse. Liver disease, diabetes, inflammation of the pancreas, and gallstones also occur in some people with cystic fibrosis (3).
   - In men - CF leads to azoospermia and is a reason for male sterility.

II. Laboratory confirmation of clinical diagnosis - The sweat chloride analysis is recommended as the diagnostic test for CF. The sweat test measures the amount of salt (sodium and chloride) in sweat. Normally, sweat on the skin surface contains very little sodium and chloride. People with CF have 2 to 5 times the normal amount of sodium and chloride in their sweat.

A sweat sample is collected using a special sweat stimulation procedure. A sweat-stimulating liquid (pilocarpine) is applied to a small patch of skin on the arm or leg. An electrode is then placed over the site and a weak electrical current stimulates the area. After several minutes, the area is cleaned and sweat is collected for about thirty minutes, either into a plastic coil of tubing or onto a piece of gauze or filter paper. The sweat obtained is then analyzed.

Sweat chloride

Normal:	Less than 40 meq/l
Borderline:	40-60 meq/l
Abnormal:	Greater than 60 meq/l

The test has to be performed twice and when both results are over 60 meq/l clinical diagnosis is confirmed. In such case the patient has to be referred to genetic counseling.

III. The genetic counselor evaluates pedigree data and points those family members, who have to undergo DNA analysis. The analysis starts with the proband with clinical diagnosis CF. When it identifies mutations of CFTR gene or polymorphic markers, the family is determined as "informative" and this makes prenatal diagnosis in next pregnancies possible - secondary prevention of the disease. Identifying mutations of CFTR gene in a patient with CF makes possible testing for heterozygote status in close relatives, and when such is found - primary prevention of the disease is possible.

RESULTS AND DISCUSSION

The experience of the Genetic laboratory of University Hospital - Varna is with 31 patients with CF. The distribution according to age is seen in fig.2. As expected, most of the patients are children (94%) and only 2 patients are adults, but they have presented with CF in younger age. Obviously, mild forms of CF in adults are usually under diagnosed.

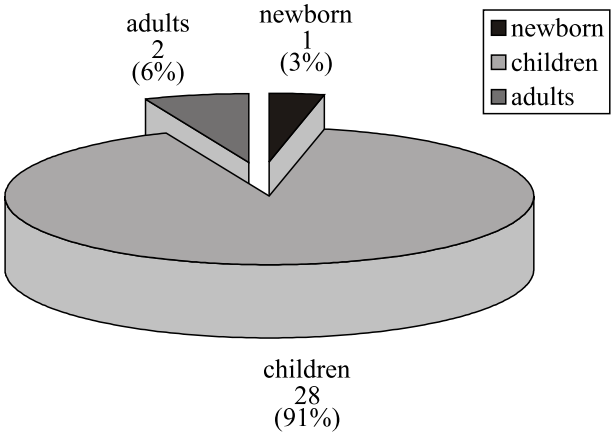

Fig.2 Distribution of CF patients according to age

DNA analysis of the CFTR gene in the CF patients, performed in the Molecular Genetic Laboratory - Sofia, found 51 mutations - 36 (70.5%) ?F508 and 15 (29.5%) other mutations. Ten of the patients were found homozygotes for ?F508 and 21 were compound heterozygotes. Two new mutations - 1717-8G-A and 1716+1 were described.

Prenatal diagnosis was performed in 8 informative families in next pregnancies, aiming secondary prevention of CF in the families. In one of these pregnancies the fetus was found to be homozygote and the pregnancy was terminated.

In terms of possibilities for primary prevention of CF in 5 close relatives of the probands DNA analysis was performed and 4 of them were found heterozygotes. The determined heterozygosity will require screening of the partners for the most common mutations of the CFTR gene. When

both partners are found heterozygotes, this will raise 25% risk for the children to have CF and is an indication for performing prenatal diagnostics and thus primary prevention will be done.

In the observed families 3 partners of confirmed heterozygotes were screened for the most common mutations of the CFTR gene and heterozygosity was not found (expected frequency of heterozygotes in the population 1:25).

In conclusion, the collaborative work of clinicians (pediatricians, pulmonologists, and gastroenterologists) and geneticists will improve the prevention of CF.

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