

Cystic Fibrosis: Correlations between Genotype and Phenotype

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Abstract

Cystic fibrosis is a clinical entity with multiple representations. Despite acquired knowledge, there is still unknown information about this disease. We tried to define the relationship between classes of mutations and clinical manifestations. We also tried to structure clinical manifestations depending on the most commonly found mutations, not minimizing intervention of environmental factors and modifier genes. We found that patients from the same family with the same mutation had different clinical manifestations, thus highlighting intervention of environmental factors and modifier genes. Diagnosis of cystic fibrosis is not easy because there are sometimes symptoms blurred, sometimes suggestive, but support the diagnosis by laboratory methods is not always possible. It is important that this condition be diagnosed as early as possible, even at birth, in order to prevent complications of the disease.

Keywords: Cystic fibrosis; Child; Genotype-phenotype

Introduction

Cystic fibrosis (CF) is an autosomal recessive inherited disease characterized by a generalized dysfunction of the exocrine glands. CF is the most common cause of severe progressive lung disease and pancreatic insufficiency in children. The severity of symptoms varies depending on the age of presentation and the different mutations of the gene [1,2].

Mutations in the Cystic Fibrosis Transmembrane Regulator (*CFTR*) gene cause CF; have been described over 1,200 mutations in the *CFTR* gene. The most common mutation is F508del.

For systematize the symptoms were attempted clinical correlations between genotype and phenotype. Intensity and the spectrum of clinical manifestations are influenced by genotype-phenotype connection, but an important role in their determinism ones is occupied by environmental factors and modifier genes.

The Sweat test measures chloride level in sweat is the standard method for diagnosing CF. Genetic testing are necessary to confirm the diagnosis of CF.

Management of CF includes the control of respiratory infection and airways mucus cleaning, nutritional therapy and treatment of complications [3,4].

Pathophysiology

Respiratory system

The reduced secretion of chlorine and increased absorption of sodium in the apical membrane of respiratory epithelial cells will cause dehydration of pericilliary mucus which will adhere to the cell surface. Adherent viscous mucus favors obstruction of excretory channels of bronchi favoring chronic bacterial colonization of injuries. The body will react by accumulation of neutrophils in the airways. Proteolytic

enzymes released by activated neutrophils, alveolar macrophages and bacteria will cause significant damage of respiratory mucosa. It has been shown that in cells with mutated *CFTR* protein it is secreted interleukin-8 in quantities of 4 times higher than in normal individuals which favors the accumulation of neutrophils in the airways. The presence of large quantities of nucleic acids produced by the degradation of inflammatory cells causes an increase in mucus viscosity resulting in persistent and worsening bronchial obstruction [4,5].

Superinfection is achieved by bacterial proliferation in the areas of stasis mucosa, with reduced drainage; thus it is a good culture medium for aggressive flora represented by *Pseudomonas aeruginosa*, *Staphylococcus aureus hemolytic*, *Haemophilus influenzae*, *Aspergillus*, *Candida*, anaerobic germs, and viruses. Respiratory impairment is conducted in 3 phases: mechanical obstruction with thick mucus, bacterial colonization, and immune phase that is related to excessive endobronchial inflammatory phenomena. In cystic fibrosis there is an imbalance between excess pro-inflammatory interleukins (ILK 1 and 8) and anti-inflammatory action (ILK 6 and ILK 10) [4].

Pancreatic disease

Pancreatic duct obstruction followed by replacement of the exocrine pancreas with fibrous tissue and fat with or without intraluminal calcifications will cause exocrine pancreatic insufficiency. The islands of Langerhans are maintained intact for long periods of time, but during the second decade of life these islands may present architectural changes by fibrous tissue [3,4].

Exocrine pancreatic insufficiency by pancreatic enzyme deficiency leads to maldigestia and malabsorption of lipids, proteins. Thus cause steatorrhea, hypoproteinemia and edema. Decreased bicarbonate secretion favors the precipitation of bile acids. By the action of jejunal microbial flora it produces deconjugation of bile salts and the formation of free acids that by poor emulsification of lipids contribute to malabsorption. Malabsorption is favored by viscous mucus secreted by the intestinal mucosa which covers the intestinal villi. It produces a

poor absorption of fat-soluble vitamins A, D, E, K, and vitamin B12 [4,5].

Pancreatic endocrine dysfunction may occur usually after the first years of life and it consists of a delayed insulin response to oral glucose load. Hyperglycemia in cystic fibrosis does not induce microvascular changes in the retina, kidney and it is not accompanied by acid-ketosis [5].

Gastrointestinal disease

Meconium ileus is caused by complete obstruction of the intestinal lumen with thick meconium, by increased content of muco-proteins secreted by the intestinal mucosa, and insufficient hydration. After the newborn bowel obstruction with fecal (bowel distal obstruction syndrome) is due to incompletely digested intestinal contents usually after meals with foods high in fat. Rectal prolapse has the following causes: bulky and sticky seats, increased intra-abdominal pressure due to frequent paroxysmal cough and a decrease in perirectal fat that supports rectum [5,6].

Hepatobiliary disease is caused by obstruction of the biliary ductules with the occurrence of cholestasis and over time biliary cirrhosis. Children at young ages may develop obstructive jaundice. Cholelithiasis occurs over time: the bile is lithogenic by high content of cholesterol, calcium bilirubinate, and proteins. Malnutrition can cause hepatic steatosis [4].

Genitourinary disease: Obstruction of epididymar duct and deferent ducts and their atrophy and fibrosis cause azoospermia and infertility.

Sweat glands: Excessive loss of sodium chloride and water especially during periods of hot weather and digestive infections causes hyponatremia, hypochloremia, and hypochloremic metabolic alkalosis.

Etiology

Cystic fibrosis gene is located on the long arm of human chromosome 7, in particular 7q31. It regulates transmembrane conductance regulatory protein synthesis (*CFTR*-Cystic Fibrosis Transmembrane Conductance Regulator) that belongs to glycoproteins P. *CFTR* protein is localized to the apical pole of various epithelial cells including airway epithelium, small pancreatic ducts, salivary glands, intestinal crypts, testicles, endometrium, renal tubules, and in the apical region but basolateral of sweat gland duct epithelium membrane. *CFTR* protein acts as a channel for the transport of chloride ions. Chloride channel is stimulated by cAMP or by its agonists but it is not influenced by ionized calcium. It has conductance, sensitivity to blockers and specific anion permeability different from the other chlorine channels. [7-9].

The *CFTR* chloride channel regulation is made by phosphorylation and dephosphorylation processes. Phosphorylation is made through some energy consuming (ATP) protein kinases which open chloride channels. Dephosphorylation is made by protein- phosphatases that close chloride channels and inactivate phosphorylation sites. The balance between the two types of enzyme activity is the main determinant of channel activity. Gene mutations encode *CFTR* protein with significant alterations in the structure, anion selective properties and the kinetics of chloride channels [7].

Various mutations can be grouped into six different classes based on mechanisms of dysfunction for the *CFTR* protein.

Class I: Defective Protein Synthesis. Mutations from this class cause the synthesis of abnormal proteins variants, unstable, with low or absent function.

Class II: Abnormal Processing and Trafficking. *CFTR* protein is incompletely glycosylated (incompletely matured) and it cannot adopt the correct spatial conformation. Therefore it cannot be detected at the cell surface. The most common F508del mutation is part of this class.

Class III: Defective Regulation. Mutations in this class affect the regulation of *CFTR* function.

Class IV: Decreased Conductance. There are several mutations that affect the properties of *CFTR* single-channel conductance. Class 4 mutations cause the synthesis of *CFTR* protein that has defective conductivity or shorter time in which chloride channel is open. Alleles in this class are usually associated with a milder pancreatic phenotype.

Class V: Reduced Synthesis/trafficking. Various mutations may be associated with a reduced amount of functional *CFTR* at the apical membrane. These mutations are associated with a milder CF phenotype.

Class VI: Decreased Stability. Mutations from this class are associated with functional but unstable *CFTR* protein. It is associated with severe CF presentation [10,11].

There is a strong relationship between CF genotype and pancreatic disease. Class I-III mutations are associated with pancreatic insufficiency, and class IV and V mutations with pancreatic sufficiency. Pancreatic sufficient patients are also at significant risk for developing pancreatitis [11].

A patient who is homozygous or compound heterozygous for class I-III *CFTR* mutations may present with mild lung disease and patients who carry at least one class IV-V mutations may present with severe disease later in life. But it is impossible to predict the severity of pulmonary disease based only on *CFTR* genotype [11]. Regarding liver disease, meconium ileus and distal intestinal obstruction syndrome, these are common in patients who carry mutations of class I-III on both alleles [11]. Class V mutations are associated with congenital bilateral absence of the vas deferens in males.

CFTR protein mutation increases the trans-epithelial potential of the respiratory mucosa. It is a loss in the permeability of the epithelium to chloride ions. Thus there is a sharp reabsorption of sodium ions and an unable reabsorption of chloride ions. These processes lead to dehydration of the mucosal glands secretions increasing their viscosity. It is also associated with a decrease in the sensitivity of the beta-adrenergic blocking agents that do not induce the secretion of chloride ions but rather may cause an increase in sodium absorption. Therefore mucous glands produce an abnormal mucus acid with a low content of sodium chloride and water promoting glandular duct obstruction. In the respiratory system sticky secretions lead to muco-ciliary system dysfunction favoring infections. Serous glands (salivary and sweat glands) secrete quantitatively normal but sodium and chlorine content is increased due to defective reabsorption in the proximal portion of the excretory canal. These qualitative changes underlie sweating test [8,9].

Epidemiology

Prevalence of cystic fibrosis varies depending ethnic groups, so it is lower in nonwhite people. At the same nonwhite population prevalence varies depending on the area of migration, possibly

through inter-population combinations. It is estimated that in the United States, CF occurs in 1:3000 Caucasians, 1:9200 Hispanics, 1:10,900 Native Americans, 1:15,000 African Americans, and 1:30,000 Asian Americans [2].

It is estimated that about 1 in 20 heterozygous white people can develop cystic fibrosis. Chance of developing CF for offspring of heterozygous parents is 25%. Prevalence of cystic fibrosis is increasing due to both uses the newborn screening and detecting people with mild disease limited to one organ system [12].

In Romania it is not yet determined the accurate prevalence, but according to the published data it not differs from the rate of the white population. In our clinic in the last 10 years we have had 21 cases diagnosed with cystic fibrosis of which most had pulmonary manifestations. Two cases of those with severe pulmonary changes died in infancy. A brother of the deceased child (offspring the same parents) developed a digestive form of the disease; he has a good evolution.

The spectrum of mutations in diagnosed cases includes the following: F508del/R1070W-3 cases, F508del/F508del-8 cases, F508del/G542X-2 cases, F508del/X-2 cases, F508del/R 334W-1 case, and unidentified mutations (X/X)-5 cases.

Clinical Presentation

Gastrointestinal symptoms may include the following: meconium ileus, abdominal distention and intestinal obstruction, increased frequency of stools, steatorrhea, recurrent abdominal pain, jaundice, gastrointestinal bleeding, and failure to thrive [2,13].

Respiratory clinical manifestations may include cough, recurrent wheezing, dyspnea, and chest pain.

Genitourinary symptoms may include amenorrhea, undescended testicles or hydrocele, and infertility.

Clinical manifestations vary depending on patient age. The average age of diagnosis is 6-8 months (Table 1).

	Respiratory system	Gut	Pancreas	Liver and bile ducts	Genitourinary system
Newborn		meconium ileus		obstructive jaundice	
The infant	recurrent pneumonia		Steatorrhea failure to thrive		
Child	recurrent pneumonia bronchiectasis nasal polyposis sinusitis pulmonary heart	Volvulus Intussusceptions rectal prolapse	failure to thrive	Gallstones Cirrhosis	
Teenager/ young adult	decreased athletic performance				azoospermia/ infertility

Table 1: Clinical manifestations according to age.

Diagnosis

The Sweat test measures chloride level in sweat is the standard method for diagnosing CF. Genetic testing are necessary to confirm the diagnosis of CF [2,13].

A value higher than 60 mmol/L of chloride is consistent with CF. A value of 40-60 mmol/L is considered borderline. In this case the test must be repeated.

For a definite diagnosis of CF genetic test positive or positive in sweat chloride test must be associated with one of the following features: chronic obstructive pulmonary disease, documented exocrine pancreatic insufficiency or positive family history [13].

Treatment

The main goals of treatment of cystic fibrosis are maintaining lung function as close to normal, maintaining an adequate nutritional status, and the management of complications [14].

The most important measures for the management of pulmonary disease include the following: chest physical therapy and postural drainage for airway clearance, bronchodilator and antibiotic therapy, adequate dose of mucolytics [15].

To maintain an adequate growth is required pancreatic enzyme supplementation, multivitamin and mineral supplements [14].

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