Cystic Fibrosis and CFTR Gene

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I- Background

- The early description of cystic fibrosis (CF) dates back to late 30s. In 1936, Fanconi identified the association between the icongenital CF of the pancreas and broncheactasis" shortly followed by Andersen who in 1938 gave the complete anatomo-pathologic description of CF.
- In 1953, Di Sant'Agnese described an excess of sodium chloride in the sweat of children affected by CF. This discovery shortly leads to the use of sweat chloride test, the only reliable diagnostic test of the disease available to this date.
- In early eighties, the abnormality of transport of salts was precisely described by Quinton (Quinton 1983) who explained the defect of permeability of chloride ions (Cl-) in the affected epithelial cells of sweat glands, and later by Knowles (Knowles 1983) who observed the same phenomenon in the respiratory epithelium.
- In 1989, the CFTR gene, implicated in the CF, was isolated. This gene is localized on 7q31 and contains 27 exons. The protein is composed of 1480 amino acids.

II- Incidence

The CF is the most common lethal autosomal recessive hereditary disorder, worst in the European origin populations, affecting an average one out of 2500 live births (= q2), i.e., one out of 25 (= 2pq) individuals is a carrier of this disease. However, this frequency varies according to geographic and ethnic origin of the patients.

III- Clinical manifestation

- The clinical presentation of CF varies among individuals from different families as well as between individuals belonging to the same family. In majority of cases the disease is diagnosed before adolescence, but some remain asymptomatic till the adult age. Clinically and biologically, it is not possible to distinguish the heterozygotes from the individuals not carrying CF mutation.
- The clinical presentation varies with age. In 10% of the affected newborns, it presents as meconium ileus (intestinal obstruction due to abnormally thick meconium). Later the symptomatology involves two major organ systems,
respiratory and digestive, manifesting as repetitive respiratory infections and signs of malabsorption.

- The respiratory involvement predominates. It is related to an obstruction of bronchioles by thick and viscous mucous favoring the growth of microorganisms. This explains the repeated respiratory infections by the opportunistic germs.
- The digestive involvement with exocrine pancreatic insufficiency (defect in the production of enzymes) is seen in 85% of the patients, leading to the obstruction of pancreatic ducts and thus lipoprotein malabsorption. The state of exocrine pancreas, PI (pancreatic insufficiency) or PS (pancreatic sufficiency), serves as a marker of the gravity of the phenotype, being serious in the former than the latter. (Kerem 1990).
- The abnormality in the sweat glands leads to an excess of sodium chloride secreted in the sweat, this loss of salt is usually responsible for acute dehydration in case of exposure to heat.
- This disease involves some other organs particularly the reproductive system and the liver.
- Atrophy and absence of vas deferens caused by obstructive azoospermia renders 98% of men sterile, on the other hand 80% of affected females are fertile.
- The hepatic involvement in 30% of cases starts as a hepatomegaly and in 9% of cases as a hepatic insufficiency. It is due to the obstruction of the intrahepatic or extrahepatic biliray tracts by compression at the level of pancreas. In 2-5% of cases this further leads to biliary cirrhosis.
- Prognosis: If not treated, the median survival rate is 3 to 5 years.
- There is no effective treatment of CF, but the timely diagnosis and symptomatic treatment has made it possible to increase the mean survival to 25 to 30 years.
- The symptomatic treatment is available in the form of respiratory physiotherapy, antibiotic therapy, nebulisation with bronchodilators and mucolytics, administration of proteases inhibitors for pulmonary symptoms and administration of substitute pancreatic enzymes and vitamins for pancreatic insufficiency.
- In advanced stages, the triple (heart-lung-liver) transplant has shown promising results but the major hindrance is the availability of donor organs.
- Among the latest therapeutic advances, the gene therapy of CF has met a number of obstacles. Other promising strategies are under study with the objective of compensating for the defect in the production and/or function of the CFTR protein depending on the type of mutation.

**IV- Diagnosis**

- The diagnosis of CF depends on the sweat chloride test. Even today it is considered to be the most reliable test for CF. The techniques used these days are simple but the quantity of sweat required is a limiting factor especially in neonates raising the risk of error to 30%.
- The values of this test can vary according to laboratories; a value of less than 40 mmoles/l of chloride is generally considered as normal.
For the range between 40 and 60 mmoles/l, the interpretation is doubtful and the test should be repeated.
For the diagnosis to be established, the sweat chloride concentration must exceed 60 mmoles/l on two separate sweat tests.
Another technique consists of measuring the bioelectrical potential difference that exists between the skin and nasal mucosa. It valuable in following cases:

1. For the early diagnosis in the neonates presenting with a suspected digestive pathology, where the sweat test is very difficult to be carried out,
2. For the confirmation of a doubtful diagnosis with some clinical signs and a borderline or negative sweat chloride test,
3. For the patient follow-up, as there is a correlation between the seriousness of respiratory involvement measured by FEV1 study (forced expiratory volume in first second) and the value of bioelectrical potential difference. The method is simple, less expensive and accessible.

V- CFTR gene and its mutations

V-1. Introduction

CFTR gene, which is responsible for this disorder, contains 27 exons spreading over 250 kb of chromosome 7 (7q31) and encodes an mRNA of 6.5 kb.

V-2. The DF508 mutation

- The most frequent mutation, a deletion of three nucleotides resulting in the deletion of phenylalanine at position 508 (DF508) is responsible for 70% of CF alleles.
- There exists a great heterogeneity in the spread of CF, there is a north-west/south-east gradient, for example 88% of DF508 cases found in Denmark and 50% in Italy.
- To explain the spread of this mutation in European population, the hypothesis of a selective advantage of heterozygotes was proposed. For example, the heterozygotes would be protected against dehydration due to diarrhea caused by enterotoxins of bacteria. The DF508 protein, associated with a decreased expression of CFTR to the epithelial surfaces, decrease the entry of pathogen into the intestinal epithelium thus providing a protection against this infection.

V-3. Spectrum of CFTR gene mutations

- Since the discovery of CFTR gene (http://www.genet.sickkids.on.ca/cftr/) more than 1200 mutations are described since the cloning of this gene, out of which 4 (excluding DF508) represent more than 2%. The frequency of certain
mutations can vary among different geographic groups (e.g., W1282X makes 48% of CF alleles among the Ashkenazi Jews and only 2% of the total CF alleles).

- The majority of molecular defects of CFTR gene are the point mutations out of which 42% are missense mutations, 24% small insertions/deletions with a frame shift, 16% nonsense mutations, 16% mutations of splicing and 2% deletion of an amino acid. Some major deletions are also reported.

- One of the particularities of CFTR gene is the existence of deleted transcripts of one or more exons among normal individuals. These transcripts are due to anomalies leading to alternative splicing out of which the most frequent and well studied is the deleted transcript of exon 9 (9-). The presence or absence of this exon is correlated with a "polymorphism" of sequence of the intron 8 situated near the acceptor site of splicing. Splicing will occur with 7 or 9T (polypyrimidine tract of intron 8) at about 90%, but when the length of this sequence is 5T then only 10 to 40% of normal mRNA will be produced, the rest of 60 to 90% will produce a mRNA without exon 9 that doesn't produce a functional CFTR protein.

V-4. Genotype-phenotype correlations

- Approximately half of the patients affected by CF are homozygous for the mutation DF508. DF508 homozygotes present a classical form of the disease with an increase in electrolytes in sweat, pancreatic insufficiency and obstructive pathology of lungs.

- The DF508 accounts for 66% of the mutations, 40% of these patients are compound heterozygotes with DF508 on one allele and another mutation of CFTR gene on the other chromosome.

- Generally, the pulmonary function, the age of onset of the disease, and the amount of chloride salt are all related to a particular genotype.

- On the other hand, the seriousness and variety of symptoms differing in the same family explain that the genotype, at the level of CFTR gene, cannot be solely held responsible for the phenotype.

- Only the mutation A455E was strongly associated with the state of pulmonary function.

- Concerning the pancreatic function the patients with one or two missense mutations have a conserved exocrine pancreatic function (PS or pancreatic sufficiency), as compared to those having two alleles of splicing mutations, nonsense or frameshift mutations and some mutations of missense which always lead to pancreatic insufficiency (PI or pancreatic insufficiency). Similarly, the patients having a PI mutation on one allele and a PS mutation on the other have a phenotype PS. With a PS mutation, the activity of the CFTR protein is sufficient for the pancreatic function.

- The effect of one mutation can be modified by a second mutation inherited in cis on the same allele.

- The polymorphism of the polypyrimidine tract of the intron 8 modifies the penetrance of certain mutations.
VI- The CFTR protein and its functions

VI-1. Structure of the CFTR protein

- The protein sequence of the CFTR is composed of 1480 amino acids.
- It consists of two repeated motifs each composed of a hydrophilic membrane-spanning domain (MSD) containing six helices and an important hydrophilic region for binding with ATP (NBF or Nucleotide Binding fold). These two motifs are linked by a cytoplasmic (R domain) encoded by exon 13, containing a number of charged residues and the majority of the phosphorylation sites (probable substrates of protein kinases A and/or C).

Figure 1: proposed structure CFTR protein, by Riordan et al. 1989 - Pascale Fanen.
Homologies exist between the primary structure of CFTR protein and members of membrane protein families, the family of ABC transporters (ATP-binding cassette), which are responsible for the active transport of substrates across the cell membrane, where ATP hydrolysis serves as the source of energy.

VI-2. Functions of the protein

VI-2.1. Cl- channel function

The early hypotheses regarding the function of CFTR protein revolved around two possibilities. The first postulated that the CFTR protein is a Cl- channel. This hypothesis was compatible with the defect in permeability of Cl- ions at the CF epithelial apical membranes. The other proposed that the CFTR protein is not an ionic channel but it plays a role in the regulation of Cl- channel either by associating with them, or by transporting a regulatory factor for Cl- channels in or out of the cell. The later results determined the function of CFTR protein as a Cl- channel.

VI-2.2. The CFTR protein, a multifunctional protein

The discoverers of CFTR gene termed it the ëtransmembrane conductance regulatorí. In fact, the CFTR protein regulates other channels also, the outwardly rectifying chloride channel (ORCC), epithelial Na+ channel (ENaC) and at least two inwardly rectifying K+ channels ROMK1 and ROMK2. Besides being a channel regulator, it also plays a role in transport of ATP, modifying the phenomenon of exocytosis/endocytosis, regulation of pH of intracellular organelles.
VI-3. Correlation of CFTR gene mutations with the Cl- channel function

The molecular anomalies have variable effects on the CFTR protein and its functions. Welsh and Smith have proposed a classification of these anomalies in relation to the Cl- channel function (Welsh & Smith 1993) (figure 3).
VI-3.1. **Class 1**: mutations altering the production of the protein. These mutations result in the total or partial absence of the protein. This class includes the nonsense mutations and those that produce a premature stop codon (anomalies of splicing and frameshift mutations). In certain cases the mutated mRNA is unstable and doesn’t produce the protein. In other cases, the abnormal protein produced will probably be unstable and degrade rapidly. This is what produces the truncated protein or the protein containing the aberrant sequence (anomalies of splicing or the frame shift). Functionally, these mutants are characterized by a loss of conductance of Cl- channel in the affected epithelia.

VI-3.2. **Class 2**: mutations altering the cellular maturation of the protein. A number of mutations alter the maturation of the protein and thus the transport of these proteins to the plasma membrane. In this way, the protein is either absent from the plasma membrane or present in a very small quantity. The mutations of this class represent the majority of CF alleles (DF508).

VI-3.3. **Class 3**: mutations disturbing the regulation of Cl-channel. These mutations are frequently situated in the ATP binding domain (NBF1 and 2).

VI-3.4. **Class 4**: mutations altering the conduction of Cl-channel. Certain segments of membrane spanning domains participate in the formation of an ionic pore. The missense mutations situated in these regions produce a correctly positioned protein that has a cAMP dependant Cl-channel activity. But the characteristic of these channels is different from those of endogenous CFTR channel with a diminution of ion flux and a modified selectivity.

VI-3.4. **Class 5**: mutations altering the stability of mRNA.

VI-3.4. **Class 6**: mutations altering the stability of mature CFTR protein.

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Citation

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URL: http://AtlasGeneticsOncology.org/Educ/CistFibID30032ES.html

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