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Original paper

CFTR GENE ANALYSIS IN PATIENT WITH ATYPICAL CYSTIC FIBROSIS

Aleksandra Nikolić[1], Aleksandra Divac[1], Nada Bogdanović[2], Marija Mitić-Milikić[2], Dragica Radojković[1]

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Summary: This paper reports a case of a patient presenting with atypical cystic fibrosis whose sweat test showes borderline values. In vast majority of cases the sweat test is essential diagnostic tool for establishing the diagnosis of cystic fibrosis, but only after the molecular genetic testing the diagnosis can be confirmed. The patient was found to be compound heterozygote for two CFTR mutations, F508del and D1152H. The presence of F508del mutation was analyzed by PSM method, while the screening for the second mutation was performed using DGGE. The strategy of mutation detection in cystic fibrosis patients, especially those with atypical presentations who carry less frequent mutations, should include both direct and indirect methods of molecular diagnostics.

Key words: atypical cystic fibrosis, CFTR gene, DGGE, molecular diagnostics

Introduction

Cystic fibrosis is one of the most common life-threatening autosomal recessive disorders that is usually estimated to affect 1 in 2000–3000 Caucasian newborns, with a carrier frequency of 1 in 26 individuals (1). In its classic and most common form, cystic fibrosis manifests with chronic obstructive lung disease, exocrine pancreatic insufficiency, elevated sweat chloride concentration and in males infertility due to obstructive azoospermia (2).

Cystic fibrosis is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, spanning 250kb at chromosomal region 7q31.3 and consisting of 27 exons. The gene was discovered in 1989, and it encodes a protein expressed at the apical membrane of exocrine epithelial cells (3). CFTR protein functions principally as a cAMP-induced chloride channel and appears capable of regulating other ion channels.

The most common mutation in the CFTR gene is F508del located in exon 10, and it is present on approximately two-thirds (66%) of all cystic fibrosis chromosomes. However, there is great mutational heterogeneity in the remaining one-third of all alleles. Nearly 1300 mutations within CFTR have been identified to date, and reported to Cystic Fibrosis Genetic Analysis Consortium (4). Although these mutations vary greatly in their frequency and distribution, the vast majority are present in either single individual or small number of individuals.

Mutations affect CFTR through a variety of molecular mechanisms which can produce little or no functional CFTR at the apical membrane. The phenotypic spectrum associated with mutations in the CFTR gene extends beyond the classically defined cystic fibrosis. Besides patients with atypical cystic fibrosis, there are large numbers of so-called monosymptomatic diseases, such as various forms of obstructive azoospermia, idiopathic pancreatitis or disseminated bronchiectasis associated with CFTR mutations uncharacteristic for cystic fibrosis (5).

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This paper reports a case of atypical cystic fibrosis that was diagnosed by the combination of direct and indirect mutation detection methods. It is shown that the patient is a compound heterozygote for two CFTR mutations.

Materials and Methods

Case history

Patient is a 38 year-old woman presented with a diagnosis of bronchiectasis. Her past medical history was noteworthy for the onset of respiratory symptoms such as: recurrent pneumonia and periods of cough and haemoptysis. Sweat test performed at the age of 6 showed borderline values. At the age of 33, computed tomography (CT) has shown the presence of bronchiectasis. At the age of 38, she presented with lung disease progression, which was observed by CT, and was referred for molecular testing for cystic fibrosis.

Methods

DNA was extracted from peripheral blood using GFX™ Genomic Blood DNA Purification Kit (Amersham Biosciences).

The presence of the most frequent CFTR mutation – F508del was detected by PCR-Mediated Site-Directed Mutagenesis (PSM) method (6). The 219bp long fragment was amplified with the following primers: 5'-GCACCATTAAAGAAAATATGAT-3' and 5'-CATTCACAGTAGCTTACCCA-3', and digested with *MboI*. Products were analyzed on 10% polyacrilamide gel.

The screening for the presence of variations in CFTR exons was performed by Denaturing Gradient Gel Electrophoresis (DGGE) method, as previously described (7). Exon 18 was amplified with the following primers: 5'-GTAGATGCTGTGATGAACTG-3' and 5'-GTGGCTATCTATGAGAAGGA-3' and sequenced with the primer 5'-TGCCCTAGGAGAAGTGTG-3' using Thermo Sequenase Cy™5 Dye Terminator Kit (Amersham Pharmacia Biotech).

Results and Discussion

The presence of CFTR F508del mutation was analyzed by PSM method. After digestion with *MboI* normal allele is digested giving fragments 202bp and 17bp long, while mutant allele remains undigested. This analysis has shown that the patient is heterozygous for F508del mutation (*Figure 1*).

The screening for the second mutation was performed using DGGE. After DGGE analysis was performed for several CFTR exons, altered band pattern was seen in exon 18. Mixing of the patient sample with control samples heterozygous for two mutations in exon 18, has shown that the mutation present in patient's sample is D1152H. Mixing with M1137V/N control gives extra heteroduplex bands, while no extra bands are seen after mixing with D1152H/N control (*Figure 2*).

The presence of D1152H mutation was confirmed by direct DNA sequencing (*Figure 3*).

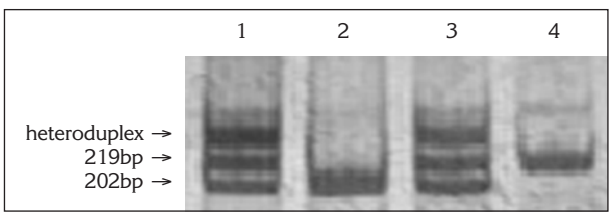

Figure 1. PSM analysis of patient's CFTR gene for the presence of F508del mutation:
1. Heterozygote for F508del
2. Homozygote for normal allele
3. Patient (heterozygote for F508del)
4. Homozygote for F508del

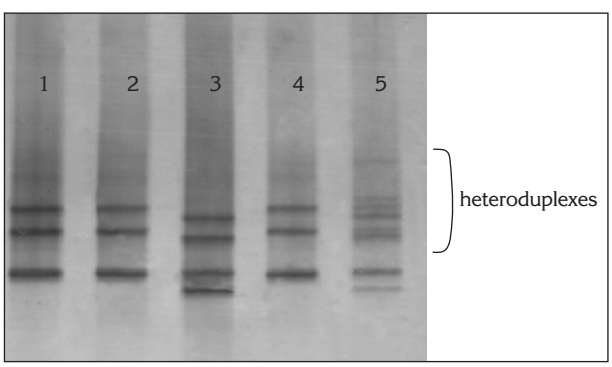

Figure 2. DGGE analysis of patient's CFTR gene for the presence of variations in exon 18:
1. patient's sample
2. control D1152H/N (heterozygote for D1152H)
3. control M1137V/N (heterozygote for M1137V)
4. mixed patient's sample and control D1152H/N
5. mixed patient's sample and control M1137V/N

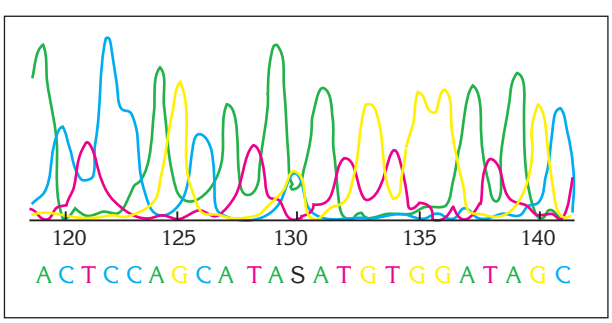

Figure 3. The part of the patient's CFTR gene exon 18 sequence containing D1152H mutation in heterozygous state (S = C/G)

The described patient, presenting with atypical cystic fibrosis, was found to be compound heterozygote for two CFTR mutations, F508del and D1152H. It has been previously reported that the F508del/D1152H genotype is associated with mild CF phenotype (8).

The CFTR mutation D1152H is caused by point mutation G to C at position 3586. Patients carrying D1152H mutation are usually diagnosed at advanced age, present mild pulmonary disease and pancreatic sufficiency. The mutation D1152H was found to be associated with normal sweat chloride values (9). Characterization at the protein and at the electrophysiological level has shown that this mutation does not alter the permeability sequence of the CFTR channels (10). However, it significantly reduces the whole cell cAMP activated chloride currents, indicating that this mutation interferes with the proper gating of the chloride channels.

In vast majority of cases, the sweat test remains the essential diagnostic tool for establishing the diagnosis of CF. Although the threshold of 60 mmol/L for the sweat chloride concentration has proven to be discriminating and useful in clinical practice, in described patient a borderline value was observed. Only after the molecular genetic testing, the diagnosis of cystic fibrosis was confirmed. In our opinion in borderline sweat chloride results, clinician should consider molecular genetics testing for cystic fibrosis. Further exhaustive genetic analysis is justified in patients with symptoms suggestive of CF and borderline sweat chloride concentration.

Although methods for direct detection of the most frequent CFTR mutations remain essential, methods for the screening of the whole gene are increasingly used for the purposes of cystic fibrosis molecular diagnostics. In our experience, the denaturing gradient gel electrophoresis is a method of choice, due to its reliability and sensitivity. It is followed by direct DNA sequencing, used for characterization of the detected aberrant pattern. The strategy of mutation detection in analysis of CF patients, especially those with atypical presentations who carry less frequent mutations, should include both direct and indirect methods of molecular diagnostics.

ANALIZA CFTR GENA KOD PACIJENTA SA ATIPIČNOM CISTIČNOM FIBROZOM

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Kratak sadržaj: U ovom radu je prikazan slučaj atipične cistične fibroze sa graničnom vrednošću znojnog testa. U većini slučajeva znojni test je glavni dijagnostički parametar za dijagnostikovanje cistične fibroze, ali se dijagnoza može potvrditi samo na osnovu rezultata molekularno-genetičkog testiranja. Utvrđeno je da je pacijent složeni heterozigot za dve CFTR mutacije, F508del i D1152H. Prisustvo mutacije F508del detektovano je PSM metodom, dok je za analizu prisustva druge mutacije korišćena DGGE metoda. Strategija detekcije mutacija kod pacijenata sa cističnom fibrozom, naročito onih sa atipičnim prezentacijama bolesti koji nose ređe mutacije, trebalo bi da uključuje i direktne i indirektne metode molekularne dijagnostike.

Ključne reči: atipična cistična fibroza, CFTR gen, DGGE, molekularna dijagnostika

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The Royal Marsden Hospital Bone-marrow Transplantation Team. Failure of bone-marrow graft without preconditioning in posthepatitis marrow aplasia. Lancet 1977; 2: 742–4.

- *Nisu navedena imena autora*

Coffee drinking and cancer of the pancreas (editorial). BMJ 1981; 283: 628.

- *Volumen sa suplementom*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxis. Pharmacol Res Commun 1988; 20 Suppl 5: 75–8.

- *Sveska sa suplementom*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988; 8 (4 Suppl): 31S–37S.

Knjige i druge monografije

- *Jedan ili više autora*

Eisen HN. Immunology: an introduction to molecular and cellular principles of the immune response. 5th ed. New York: Harper and Row, 1974: 406.

- *Urednik(ci) kao autor*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973: 12–8.

- *Poglavlje u knjizi*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA. eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders, 1974: 457–72.

- *Rad u zborniku radova*

Harley NH. Comparing radon daughter dosimetric and risk models. In: Gammage RB. Knye SV. eds. Indoor air and human health. Proceedings of the Seventh Life Sciences Symposium: 1984 Oct 29-31; Knoxville (TN). Chelsea (MI): Lewis. 1985: 69–78.

- *Disertacije i teze*

Cairns RB. Infrared spectroscopis studies of solid oxygen. Dissertation. Berkeley, California; University of California, 1965.

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  The Royal Marsden Hospital Bone-marrow Transplantation Team. Failure of bone-marrow graft without preconditioning in posthepatitis marrow aplasia. Lancet 1977; 2: 742–4.

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  Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988; 8 (4 Suppl): 31S–37S.

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  Harley NH. Comparing radon daughter dosimetric and risk models. In: Gammage RB. Knye SV. eds. Indoor air and human health. Proceedings of the Seventh Life Sciences Symposium: 1984 Oct 29–31; Knoxville (TN). Chelsea (MI): Lewis. 1985: 69–78.

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