Regular genetic kit for CF mutations detection

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Background: A characteristic aspect for Romania is the CF mutations heterogeneity which leads to a reduced percentage of genotype identification.

Objectives: Assessment of the efficacy of a mixed panel for CF mutation detection in Romanian patients.

Methods: We evaluated retrospectively 40 patients(pts) with typical CF, registered in the National CF Center Timisoara. The genetic tests were performed using a mixed panel – (29 mutations – panel 1) – ARM and another kit for 38 mutations (panel 2) – PCR. 18 mutations were common to the two kits; the total number of identifiable alleles was 49.

Results: The first panel identified the following mutation, in order of frequency: AF508, G542X, N1303K, 621 + 1 G→T, I148T, representing 17.2% from panel 1 mutation. We found the following patients genotypes: 21 pts with F508del/F508del, 10 pts with F508del/G542X, 5 pts F508del/G542X, 1 patient F508del/N1303K. In 3 pts with compound genotype non-F508del (I148T, N1303K or G542X), the other allele could not be identified, complementary genetic testing done in parents have ruled out the possibility of homozygous genotype for non-F508del. In 13 patients (32.5%) we could not fully identify the genotypet, thus they were further tested with panel 2.

Conclusions: Correspondence of kits with identified mutations in CF Romanian patients is low, although kits contain the most frequent mutations used in Europe. Genetic heterogeneity in Romania limits significantly the possibility of detection of both alleles, the diagnosis rate of heterozygote being reduced. The question of using additional kits or methods like CF gene sequencing raise the issue of a very high cost.

A novel StripAssay for the detection of cystic fibrosis mutations in the Turkish population

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Cystic fibrosis (CF) is among the most common life-threatening autosomal recessive disorders, with an estimated incidence of approximately 1 in 3500–4000 live births in Caucasians. The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Worldwide, the most frequent mutation F508del accounts for 18–87% of CF chromosomes depending upon ethnicity. This mutation decreases along a northwest to southeast gradient, and shows a frequency of only around 25% in the Turkish population. In general, a very high heterogeneity in pathogenic CFTR mutations has been reported in Turkish patients.

We have developed a reverse-hybridization assay for the rapid and simultaneous analysis of 24 CFTR mutations, as well as the IVS8 polyT (5T/7T/9T) variants. The CF StripAssay TUR shows a coverage of around 60% of mutations found in the Turkish population, which is more than any other commercial CF test currently available. The assay is based on multiplex DNA amplification and hybridization to teststrips containing allele-specific oligonucleotide probes for each mutant and wild-type allele. The procedure is rapid, simple and convenient, accessible to automation and requires very small amounts of samples, which is of particular importance for prenatal diagnosis and newborn screening.

Spectrum of CFTR mutations in Polish cystic fibrosis patients

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Background: Identification of both mutated CFTR allele in patients is extremely important for early CF diagnosis, genetic counseling and patient specific treatment.

Patients and Methods: We selected 62 Polish CF patient presenting clinical features of cystic fibrosis (elevated sweat chloride >60 mmol/L, visibly bronchopulmonary disease, peribronchial alterations, abnormal chest X-ray) in whom at least one mutated CFTR allele was unknown, after initial screening using INNO-LiPA CFTR19 and INNO-LiPA CFTR17+Tn kits. In the next step of our diagnostic procedure we sequenced whole 27 CFTR exons together with exon/intron junctions followed by Multiplex Ligation-dependent Probe Analysis.

Results: We identified 43 different mutations, representing 98% of CF chromosomes, including 7 novel CFTR mutations (3600→1G→T; 341_353del13bp; 13_15delCCT; 80G→T; 1853_1863del11bp; 1811→90C>A; 4035_4038dupCCTA). A significant proportion (31/43) of the different mutations identified would not have been detected by the recommended kits. The 39% of the mutations (17/43) occurred with a relative frequency >1%, which illustrates that the identified mutations are not all rare.

Conclusion: Variety of existing changes in Polish CF patients population cause that the large quantity of them are not included in routine diagnostic panel, thus some changes may escape detection. This make diagnostic problem, especially during newborn screening procedure when identification of both mutated CFTR allele is very important to confirm CF before the first symptoms appeared. Recognition of the most frequent mutation in Polish population will help to design DNA testing strategies.

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Detection of the mutation D1152H in the CFTR gene in University Hospital Brno, Czech Republic

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For the DNA analysis of CFTR gene we use since autumn 2009 the beginning of newborn screening of cystic fibrosis (CF in CR) the kit Elucigene, which allows the detection of 32 or 50 mutations of CFTR gene including the mutation D1152H. Newborns numbered 84 485 were born and looked through for CF in the Moravian part of the Czech Republic in two years period (1.12.2009 to 31.12.2011). The mutation D1152H in CFTR gene was the second most frequent CF mutation we detected in newborns.

The DNA analysis of CFTR gene was performed in 953 newborns and we found 7 alleles with the mutation D1152H in CFTR gene – 6 healthy heterozygotes and one compound heterozygote with CFTR genotype: [N1303K] + [D1152H] without clinical manifestations of cystic fibrosis and with the normal value of chloride in sweat in newborn age.

The phenotype associated with mutation D1152H with other CFTR mutation in the trans position, there are only a very limited knowledge (phenotypic characteristics: chronic sinusopulmonary disease, bronchiectasis – about 70%, Pseudomonas colonisation – less than 30%, the majority are pancreatic sufficient, the disease is diagnosed on average at the age of 30 years.

Our adult patients with CFTR genotype: [F508del] + [D1152H] corresponds to these phenotype, the diagnosis of CF was established in 2011. In male carriers of the CFTR mutation D1152H we confirmed an origin by determining the Y-chromosome haplogroup, a group of Y-chromosomes related by descent from a set of STR markers PowerPlex® Y.

We analysed 7 male individuals and identified two Y-chromosome haplogroups both descendent from West Europe in D1152H carriers and an individual with Jewish descent.