46 Simultaneous detection of common cystic fibrosis mutations by reverse-hybridization teststrips

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Cystic fibrosis (CF) is one of the most common autosomal recessive disorders, with an incidence of approximately 1 in 3000 live births in Caucasians. CF is caused by mutations in the cystic-fibrosis transmembrane regulator (CFTR) gene, encoding a chloride channel protein. Patients with classical CF accumulate viscous mucus in the respiratory and gastrointestinal system, leading to chronic lung infections, excess salt loss, difficulties in digestion, and ultimately to a shortened life expectancy. More than 1000 CFTR mutations have been described to date, the majority being very rare or private. The most frequent mutation worldwide F508del accounts for 30–72% of CF chromosomes depending upon ethnicity. Overall there is great heterogeneity in the remaining pathogenic mutations, as type and distribution vary substantially between populations.

We have developed a reverse-hybridization assay (Cystic Fibrosis StripAssay) for the rapid and simultaneous analysis of common CFTR mutations. The assay covers 23 mutations recommended by the ACMG plus 10 additional ones prevalent in different parts of Europe, as well as the IVS8 polyT (5T/7T/9T) variants. Thus a coverage of 70–93% can be obtained almost all over Europe. The test is based on multiplex DNA amplification and hybridization to teststrips presenting a parallel array of 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. Currently the Cystic Fibrosis StripAssay is validated in a multi-center study.

47 CF diagnostic challenges: sweat test and CFTR gene mutation testing results

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Since 1998 sweat tests has been done among 810 CF suspecting children and 45 adult men with idiopathic spermatogenesis failure. The molecular genetic testing of CFTR gene mutation was performed among 470 persons with CF suspicion. 145 CF probands were detected. Nineteen different CFTR gene mutations were found and about 85% of CFTR alleles have been identified. Chloride concentration in sweat was in range from 7.8 to 108.2 mmol/l and 16.7% of analysis were positive. Among children with positive sweat test in 71% patients two CFTR gene mutations were detected, in 21% – one mutation and in 7.6% – no one mutations has been identified. No one case of pseudo positive sweat test results we have had. Among children with normal sweat test range one case of F508del heterozygous was identified: 3 years boy with maldigestion and without pulmonary disorders. This patient is under observation but diagnosis of CF was not confirmed. Among adult men with idiopathic spermatogenesis failure in 60% cases sweat test was normal (<40 mmol/l), in 33% borderline and in 6.1% (>60 mmol/l) positive. CFTR gene mutations analysis revealed F508del mutation and 5T allele in 5% of patients with normal and in 18% of patients with borderline sweat test. 2 man with high level of chloride concentration (79.5 and 90.1 mmol/l) have no CF symptoms except aspermia. One of them is heterozygous for F508del, other one is negative for all analyzed CFTR mutations. The results have shown insufficiency of the revealing the CF cases (~13 per year to 40 expected) and need of CF neonatal screening. Sweat test is important for Cystic Fibrosis diagnosis establishing as well for CFTR related diseases identification.

48* Parthenogenetic activation of human oocytes as a model of polar bodies PGD feasibility for CF

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Pre-implantation genetic diagnosis (PGD) on polar bodies and/or blastomeres has become an established clinical approach for prevention of genetic disorders as CF. We evaluated a model of parthenogenetic activation (PA) of human oocytes in order to assess the feasibility of CF-PGD in our laboratory, using an experimental tool independent from fertilization. After partial zona pellucida dissection, first polar bodies (PB1) were removed from spare donated metaphase-II oocytes and transferred to lysis buffer. PA was conducted on biopsied oocytes through exposure to ionomycin in culture medium. After 18 hours oocytes showing one pronucleus and the second polar body (PB2) were considered activated: PB2s were biopsied and treated as PB1s. PGD molecular procedure involved a fluorescent multiplex PCR analysis of highly polymorphic short-tandem repeat (STR) markers, to identify the haplotype associated with the maternal mutation. A panel of 10 STR markers flanking CFTR gene were selected to ensure sufficient informativity in all cases. Cases with less than three informative markers were excluded. 21 oocytes were selected from seven women. 20 of them were subjected to PA, and 15 oocytes extruded the PB2. Three out of 15 PB2 did not get any amplification product. Out of 12 PB1 analyzed, 10 were heterozygous and 2 homozygous for the markers evaluated, thus resulting in a percentage of recombination of 83%. We observed a frequency of allele drop out (ADO) of 5.1% (3/59). The model of PA confirms that PGD for CF is feasible; however, in our study, the high recombination in CFTR gene limits to 17% the possibility to perform PGD using only the PB1, according to the Italian legislation.