

**15 Frequency of mutations of the cystic fibrosis transmembrane regulator (CFTR) gene in a cohort of consecutive patients candidate for assisted reproductive techniques**

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**Objectives:** An association between cystic fibrosis transmembrane regulator (CFTR) gene mutations and infertility may occur. This study investigated the frequency of mutations in the CFTR gene, in a group of consecutive patients candidate for assisted reproductive techniques with the aim of identify subjects carriers of the most severe ones.

**Methods:** We screened 11208 healthy subjects (5943 females and 5265 males) for 56 CFTR gene mutations and IVS8-poliT polymorphism utilizing the CFTR INNO-LiPA Amplification kit including both general and Italian regional strips. The frequency of mutations were separately calculated and the Chi-square test was used for comparisons.

**Conclusions:** CFTR mutations were detected in 6.2% of the patients, a percentage similar to that reported in the general population. The most common mutation was  $\Delta F508/N$  observed in 0.9% of patients. No difference in the gender distribution was evidenced. In the large group of patients analyzed 87.7% were wt, 11.8% carrier of one mutation, and interestingly 0.5% compound heterozygous.

Our data support the relevance of an accurate determination of mutations in the CFTR gene in order to inform the couple of their carrier risk and the possibility on having affected child. Moreover, our findings highlight the potential of genetic screening as a tool to identify possible compound heterozygous subjects without CF-like symptoms.

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**16 Newborn screening for cystic fibrosis in Switzerland – Consequences after analysis of 4 months pilot study**

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**Background:** Newborn screening for cystic fibrosis (CF), based on immunoreactive trypsinogen (IRT) and 7 most common CFTR mutations, was introduced in Switzerland on January 1<sup>st</sup>, 2011. The suggested IRT value for the 99<sup>th</sup> percentile from the literature was 60 ng/ml. In a test run in December 2010, the corresponding IRT value for the 99<sup>th</sup> percentile turned out to be lower (45 ng/ml). One aim of this pilot study was to find out if this cut-off is too low and resulting in unnecessary 2<sup>nd</sup> IRT measurements if no CFTR mutations were found.

**Methods:** We evaluated the IRT cut-off, recall rate, the number of children referred to a CF centre, and compared it to IRT values of the confirmed diagnosis. All children referred to a CF centre had an additional IRT measurement when sweat testing was performed.

**Results:** In the first 4 months, 27,297 IRT tests were performed, 0.92% of them (251/27,297) were above 45 ng/ml, and DNA screening was performed. In 211 children, a 2<sup>nd</sup> IRT measurement was necessary (recall rate 0.77%). 40 children were referred to a CF centre (referral rate 15.9%) and the positive predictive value was 20% (8/40). No child with CF had an IRT value <50 ng/ml. All 15 children with initial IRT between 45 and 60 ng/ml and no CFTR mutations had a normal 2<sup>nd</sup> IRT test. After changing the IRT cut-off, the recall rate decreased by 45%. In the further 8 months, only 228 children needed a 2<sup>nd</sup> IRT test and 45 were referred to a CF centre for sweat testing, and only 4 out of them had twice an elevated IRT value but no CFTR mutation.

**Conclusions:** The change of the IRT cut-off from 45 to 50 ng/ml (99.5<sup>th</sup> percentile) resulted in less recalls without losing diagnostic accuracy.

**17 Pilot newborn screening program for cystic fibrosis in Uruguay: IRT-PAP**

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**Introduction:** Since June 2010, the Public Health Ministry in Uruguay decreed mandatory the newborn screening for Cystic Fibrosis (CF). Our algorithm is to perform the immuno-reactive trypsin (IRT) in dried blood spot. If this IRT value is higher than the cut off we request another sample which must be collected before 30 days of baby's life. However, the second sample does not come on time to perform the IRT so we started a pilot program with a second marker: pancreatitis-associated protein (PAP). PAP is done to all first sample with IRT elevated as well as to every second sample.

**Objective:** To present the results of our newborn screening of CF in the pilot program.

**Materials and Methods:** The samples used were dried blood spots on Whatman S&S 903 filter paper, the first one is taken with 40 hours of life. The methods used are IRT Quantase enzyme immunoassay BIORAD and Mucopap enzyme immunoassay DYNABIO optimized.

**Results:** From April to November 2011 we processed 32650 samples. 289 samples were high for IRT, to this samples we performed PAP and 57 of them were positive. These patients were called to confirm the CF by sweat test and molecular analysis. 7 patients were confirmed by both methods. Positive predicted value (PPV) was 2.4% for IRT-IRT algorithm and increased to 12.3% with IRT-PAP algorithm.

**Conclusions:** With IRT-PAP algorithm we have a specific and sensitive program for the detection of CF. One of the advantages of this marker is that is useful independently of the time the sample is collected, which give us more time to define the diagnosis of the patients. With this algorithm we believe we can solve problem related to the delay of the second sample.

**18 Two years experience with CF newborn screening in University Hospital Brno**

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**Objectives:** Newborn screening (NBS) for cystic fibrosis (CF) has been implemented in the form of nationwide Immunoreactive trypsinogen (IRT) / DNA/IRT scheme in the Czech Republic since autumn 2009. Total number of 84485 children were born and examined for CF in the Moravian part of the Czech Republic in two years period. The aim was to evaluate incidence of CF and frequency of pathologic alleles in CFTR gene.

**Methods:** Dry blood spot was checked for IRT. Levels above 65 ng/ml were sent to molecular analyses, where they were screened for 50 mutations. Children with only one mutation were invited for sweat test.

**Results:** Elevated IRT was detected in 953 cases out of the total 84585. Thirteen CF children were diagnosed. All of them were symptomatic already in 6 weeks. After adequate therapy, all of them are in very good status. Four children were heterozygous for two mutations but with sweat test under 30 mmol/l and healthy. We detected combination of F508del/R117H twice, and both F508del/D1152 and N1303K/D1152H once. Another 78 children with one mutation were checked, all were healthy.

During the last two years we diagnosed one CF in 6020 newborns. There were only 14 types of pathologic alleles. F508del was the most frequent mutation in 58% followed by D1152H and R117H.

One baby passed NBS undetected with IRT 61 ng/ml respective 40 ng/ml and was diagnosed as CF homozygous F508del because of clinical symptoms later at the age of 8 weeks.

**Conclusions:** We can confirm that NBS is a valuable method; all CF children diagnosed at early age are doing well. In symptomatic babies with negative NBS it is necessary to perform sweat test as well. Failure of NBS is possible.