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Neonatal Screening for Cystic Fibrosis in Republic Sakha (Yakutia)

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ABSTRACT

Neonatal screening for cystic fibrosis involves early presymptomatic diagnosis of the disease in newborns. The aim of the study was to determine the effectiveness of neonatal screening for cystic fibrosis in the Republic Sakha (Yakutia). The results of neonatal screening, sweat samples and molecular genetic testing of DNA samples from 126 newborns hypertripsinogenemia (level of immunoreactive trypsin in the first test – IRT>70 ng/ml) and 120 patients to the most common gene mutation *CFTR*. Mutations were found in 17 individuals (11 - heterozygotes, 6 - homozygotes).

Keywords: cystic fibrosis, neonatal screening, sweat test, gene, *CFTR*, DNA-diagnostics

INTRODUCTION

Neonatal screening for cystic fibrosis (CF; OMIM no.219700), is one of the most effective methods, which provides early detection of the disease and prompt initiation of treatment to prevent or slow down development of severe manifestations of the disease leading to disability and/or early death of a child. Cystic fibrosis has now become a national priority program in the Russian Federation. Sakha Republic (Yakutia) in 2006 held mass screening of newborns for CF as part of the national "Health" priority project, along with phenylketonuria, galactosemia, congenital hypothyroidism and congenital adrenal syndrome were all included in the list of hereditary diseases subject to mandatory newborn screening.

Increased levels of immunoreactive trypsin (IRT) in blood plasma of patients with CF were discovered in the 1970s; and this was a beginning of mass newborn screening for this disease. Screening protocol for CF Russia includes 4 stages: determination of IRT in newborn dried blood spot, IRT repeat (retest), sweat test and DNA-diagnostics, and only three are required [2].

Cystic fibrosis (CF) is the most common hereditary multiorgan pathology characterized by pronounced genetic heterogeneity and clinical polymorphism. This monogenic disease is caused by mutations of the *CFTR* gene (cystic fibrosis transmembrane conductance regulator), characterized by lesions of the exocrine glands vital organs and usually requiring a severe

rehabilitation course and prognosis [1]. Currently more than 1500 described mutations and 250 polymorphisms in the *CFTR* gene (*CFTR* mutation database, http://www.genet.sickkids.on.ca/cftr/). *CFTR* gene was isolated in 1989. It contains 27 exons, spans 250000 nucleotide pairs and is located in the middle of the long arm of chromosome 7. A multicenter study involving local scientists (N.I.Kapranov, E.K.Ginter, V.S.Baranov) conducted in 1999-2000, has covered 17 countries in Central and Eastern Europe, including Russia. As a result of these studies a list of 33 common mutations specific to those countries was suggested. Among them, the most frequent mutation is delF508; second in frequency - del21kb (*CFTR* dele 2.3). Frequency of the next 6 mutations (N1303K, G542X, W1282X, 3849+10 kbC> T, 2143delT, 2184insA) exceeded 1% [3]. The frequency of cystic fibrosis varies in different populations of the world very widely (for example, in Europe from 1:1800 births in Ireland to 1:26000 in Finland). Estimates of the frequency of cystic fibrosis in different populations of the Russian Federation are also quite different (from 1:4900 to 1:12000 live births) [4].

MATERIALS AND METHODS

Material for the study is based on the data of a survey on the CF 112 019 newborns in the period 2006 - 2013. Determination of immunoreactive trypsin (IRT) level in dried blood spots was performed by immunofluorescence method with a time resolution using reagents DELFIA Neonatal IRT (Perkin Elmer/Wallac, Finland). The study was conducted in newborn blood biochemical laboratory Genetic counseling Perinatal Center Republican Hospital №-1 - National Medical Center. Positive result of screening (IRT>70 ng/ml) served for re-determination of IRT in blood samples. High-level re- IRT (>40 ng/ml) served for a sweat test. Sweat test was carried out by titration Gibson - Cooke Sweat and using aNanoduct (Wescor, USA) analyzer (system to stimulate perspiration and sweat analysis). Measured "equivalent" concentration of sodium chloride in the sweat fluid. Accepted as the norm results 0-60 mmol/l, figures 60-80 mmol/l were considered borderline, 80 mmol/l or higher were considered positive for cystic fibrosis. As part of neonatal screening in the molecular genetics laboratory in 2011 initiated molecular genetic testing for cystic fibrosis *CFTR* gene using the test system CF-11 (mutations del21kb, L138ins, delI507, delF508, 394delTT, 604insA, 1677delTA, 2143delT, 2184insA, 3821delT, 3944delTG) and CF5-L (G542X, W1282X, N1303K, 3849+10kbC>T, R334W), developed by the "Center of molecular Genetics", Moscow. Isolation of DNA for molecular genetic studies performed from venous whole blood and blood spots on filter paper, using a reagent kit «DNA-Prep» when

dealing with blood stains and «DNA-Blood» on liquid blood, according to the method recommended by the manufacturer.

Since December 2013 a diagnosis of CF by reverse hybridization on the microarray with a set of "Cystic fibrosis - biochips" ("Alcor Bio", St. Petersburg) was introduced Detection of hybridized microarray performed on PerkinElmer ScanRi microarray scanner (PerkinElmer, USA). The method allows to detect 25 most frequently occurring in the territory of the Russian Federation of mutations in *CFTR* at the same time: F508del, Delex2-3, 2143delT, G542X, G551D, 2184insA, W1282X, N1303K, 3732delA, 1717-1G> A, 1677delTA, 2188AA-G, S1196X, 3821delT, R553X, 1078delT, I507del, 2789 +5G>A, R1162X, 3849+10kbC>T, G85E, 621+1G>T, R347P, R347H, R334W. The current method is being optimized.

RESULTS AND DISCUSSION

Mass screening for cystic fibrosis was launched in August 2006. During this time, were examined 112019 births (screening coverage - 99.5 %). The number of newborns with initially elevated levels of IRT - 1474 (1.3%), held on 21 - retest the 28th day of life - 1050 (71.2 %). Improving IRT at retest - 52 (5 %).

Gibson-Cooke sweat test method was held since 1989 in biochemical laboratory;the sweat test titration performed about 200 patients annually. The Nanoduct analyzer was used since 2008 and has been used in 239 cases (we tested both children with a positive retestand children with a single positive test, which for some reason has not been retested).

Four children with CF were revealed according to the results of the sweat test. One child from Yakutsk, 3 - from rural areas: Tomponsky, Mirnynsky and Aldanskyregions. Meconium ileus has occurred in one case. We identified patients with children at the first level of IRT survey averaged 184.5 ng/ml. During the retest, patients IRT level ranged in average 160.8 ng/ml. Thus in this form of screening, there was a direct correlation between degree of increase of a biochemical marker and a share of diagnosed patients.

Molecular genetic study in 2011 was conducted with 246 patients. Homozygous for the mutation delF508 found in 4 patients, heterozygous state in 11, and compound del21kb/W1282X del21kb/2184insA - 2 cases. One child died with IRT > 200 ng/mL, sweat test is not carried out. The diagnosis of cystic fibrosis was confirmed by molecular genetic studies have identified mutations in the homozygous state delF508.

The results of molecular genetic testing for the period 2011-2013

Year	Number of the investigated	Genotype	Number of identified
2011	24	delF508/-	3
		delF508/delF508	2
		del21kb/W1282X	1
2012	168	delF508/-	6
		delF508/delF508	1
		del21kb/2184insA	1
2013	54	delF508/-	2
		delF508/delF508	1

As a result of neonatal screening, patients affected by CF in most of the cases are diagnosed before the onset of clinical manifestations (except for individuals who have CF manifests meconium ileus). A link between *CFTR*-genotype and phenotype in CF is not direct. Clinical manifestations of CF are a consequence of interaction of three factors: *CFTR*-mutations modifying factors in the *CFTR* gene and/or other genes and environmental influences [5]. Classification of *CFTR*-mutations based on the nature of the molecular defect and its impact on the function of chloride channels. Class I, II, and III mutations, such as W1282X, delF508, G542X, significantly reduce or completely destroy activity of *CFTR*-channel and associated with classic CF phenotype: lung disease, elevated levels of chloride in sweat, pancreatic insufficiency and impaired fertility in men. When mutations IV, V and VI classes, such as R334W and R117H, partly chloride channel activity is still present, although reduced chloride conductance. These mutations are associated with preservation of pancreatic function and late-onset disease. However, it should be borne in mind that nature and severity of lesions varies widely even in patients with the same genotype [6].

CONCLUSION

Neonatal screening is the only method of early diagnosis of cystic fibrosis that allows early treatment and improves the quality and life expectancy of patients with cystic fibrosis. Neonatal screening is onlyeffective if the coverage is not less than 98 % of newborns;the test forms are timely delivered to the laboratory and supply of reagents and consumables is uninterrupted. Use of confirmatory techniques such as sweat test for Gibson-Cooke, sweat

analyzer type Nanoduct and molecular genetic methods are requiredfor a complete verification of the diagnosis.

Given the complexity and sensitivity of the test sample, the laboratory performing the test must meet certain requirements: it must conduct more than 150 tests per year. The method has high requirements for qualification of performing personnel and quality of applied reagents. At the same time, the titration method does not require special equipment and consumables. This classic method is the "gold standard" for diagnosis of cystic fibrosis. For the first months of life, which are difficult or impossible to obtain sufficient sample of sweat on the filter paper for a reliable analysis, the Nanoduct analyzer is a choice method. The analyzer also provides an opportunity for a sweat test outside the laboratory.

Today, the most relevant molecular genetic methods for the diagnosis of cystic fibrosis are multiplex PCR and hybridization on microarray that simultaneously detect a number of mutations in *CFTR*. Molecular genetic methods allow us to confirm the diagnosis of cystic fibrosis in borderline sweat test results above. Value of molecular genetic diagnosis is also in getting more information for research. The disadvantage of molecular genetic techniques is the high cost and inability to identify unintended mutations used method. Whereas conducting a sweat sample test can reveal near-negative, as well as a small percentage of false-negative case results, this requires conducting DNA diagnosis of cystic fibrosis.

Introduction of molecular genetic analysis of the *CFTR* gene identified in newborn screening patients has revealed the type of mutation delF508, del21kb, W1282X and 2184insA in the population of the Sakha Republic (Yakutia).

Diagnosis of the disease through neonatal screening allows early treatment and rehabilitation activities that should lead ultimately to improve the quality and duration of life of these children. Genetic analysis to the manifestation of clinical manifestations needed for preventive measures and for the prognosis of the disease. Research findings are important for planning and implementation of programs for early diagnosis, treatment and rehabilitation of children with cystic fibrosis.

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