

REPORT

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Since 23 October till 10 November 2008 I have been having the scientific fellowship under the sponsor of Eurogentest. I have acquainted with the organization all stages of molecular-genetic testing process at the Division of Clinical Molecular Genetics and the National Cystic Fibrosis Centre University Hospital Motol, Charles University, Prague.

I have been studying the problem of molecular-genetics diagnostics of hereditary diseases since 1994. I am a senior research assistant of the research group which perform molecular-genetics testing of CFTR, Phenylketonuria, Nijmegen breakage syndrome (NBS), Dystrophy, ATM, SMN1 gene mutations, Y-chromosome microdeletions and MTHFR, MTR, MTRR, IL-10, VDR-3 gene polymorphisms. The methods we used at the laboratory allow confirming the presence or absence of proper mutation (RFLP, heteroduplex, deletion analysis, ARMS et.al.).

This fellowship gave me the possibility to learn many new sensitive techniques to found and identify mutations of different genes: DNA sequencing using an automated ABI sequencer, high-resolution DNA melting, MLPA.

I paid attention to learn the organization of molecular diagnostic laboratory according to Eurogentest requirements. The opportunity to be familiar with different apparatus used in the laboratory is very important for me for planning and choice future purchase of laboratory equipments.

The training was focused on analysis of CFTR gene mutations. Also I had chance to analyse samples of CF patients with unidentified mutations using the method I learn. I have been doing all steps of the DNA sequencing and high-resolution DNA melting analysis.

Previous results. CFTR mutations' testing was performed on 980 CF suspected persons and their relatives since early 1990s. The diagnosis of CF was established in 124 patients basing on family history, typical clinical symptoms, elevated sweat chloride concentration and DNA analysis data. Based on the frequency and spectrum of the most common CFTR mutations in Western Ukraine, we developed a protocol for practical CF diagnostic purposes. In the study group the following percent of mutant CF alleles was established: F508del – 54.50, N1303K – 4.05, G542X – 3.15, CFTRdele2,3 – 3.15, W1282X – 2.70, 3849+10kbC/T – 1.21. Mutations 1898-1G>A, 3272-1A>G, R334W, 1717-1G>A, R553X, 2183AA>G, 621+1G>A, 2721del11, R347H were encountered at a frequency less than 1%. The majority of patients have one identified F508del mutation with the other unidentified allele. Our results confirm the necessity of performing additional analysis of DNA samples with unidentified CF alleles in order to establish other specific mutations, which, similar to CFTRdele2,3(21kb), are widely distributed in CF patients of Slavic origin but are not found in other populations.

The study of CFTR gene mutations of patients from West Ukraine allowed receiving concerned preliminary results which have to be the object of the future scientific projects.

1. Mutation 2184insA was found in 15 patients (including two homozygous). We discovered 2184insA mutation with the frequency 6% is the second after F508del most common allele in our population. This mutation is not included in standard CFTR gene mutations panel.

2. We identified one new CFTR mutation (in exon 7) which was not included in the CFTR data base.

I am keen to elaborate all these methods at the Institute of Hereditary Pathology of Academe of Medical Sciences of Ukraine. I am very grateful to Prof. Milan Macek, Jr for his kind support and guidance. My special thanks to Alexandra Stamberгова and

Petra Krenkova and all the technicians, Ph.D students and medical doctors for teaching me the techniques I require to know and for their help and support. I am very grateful to Eurogentest for giving me opportunity to have this fellowship.