Update of CFTR mutation spectrum in cystic fibrosis patients from Peru

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Objectives: Reliable data on the spectrum of CFTR gene mutations in populations where the disease is unknown, are difficult to obtain. The aim of this study was to analyze the CFTR gene in cystic fibrosis patients, affected by CF and referred to CF Centers of Hospital Nacional Edgardo Rebagliati Martins and Instituto Nacional de Salud del Niño (INSN) of Tumbes (Peru).

Methods: 32 patients were included in the study. DNA samples were first tested for the 30 frequent mutations covered by the Elucigene CF30 kit (GeneProbe Diagnostics), followed by comprehensive CFTR coding sequence analysis by Next Generation sequencing on a PGM (Ion Torrent) using an Ampliseq CFTR optimized panel (Life Technologies).

Results: As expected, the frequency of the F508del mutation is low, representing only 26.5% of the CF alleles. Among the other 15 identified mutations, only 6 [p.G542X (7.5%), c.3140−26A>G (4.5%), c.2657+5G>A (3%), p.I507del (1.5%), p.R334W (1.5%), and p.R1162X (1.5%)] are part of the Elucigene kit and represent 12.3% of alleles. Interestingly, after the second mutation p.G542X, which represent 7.5% of alleles, three rare or novel mutations, c.739_742dup in exon 6 (4.7%), p.P499L in exon 11 (4.7%) and p.L571S in exon 13 (3.1%) were frequent in this population. Finally, nearly 70% of the alleles were characterized.

Conclusion: These preliminary results, obtained on a small population of CF patient from Peru provide important data on the CFTR gene mutation spectrum in this population and are important for diagnosis and genetic counseling.

Application of next-generation sequencing for the analysis of CFTR in Serbian CF patients

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Objectives: Our laboratory has a longstanding history of CFTR gene analysis. In this study we applied next-generation sequencing (NGS) technology to look for CFTR sequence variants in samples diagnosed as CF but in which one or no mutation was identified after analysis of 7 most common mutations (c.1521_1523delCTT, c.489+1G>T, c.1624G>A, c.1657−5G>A, c.1585−1G>A, c.3909C>G) or after sequencing of the whole coding sequence of CFTR gene.

Methods: Library pool of 24 CF patients with a positive or borderline sweat test was generated using multiplex PCR amplification (MASTR, Multiplicom) followed by sequencing on a MiSeq instrument (Illumina). Clinical relevance of detected CFTR variations was assessed based on the CFTR database (http://www.genet.sickkids.on.ca/app) and CFTR2 website.

Results: Adequate sequencing coverage provided accurate detection of CFTR variants. The NGS sequencing data correctly confirmed 18 germ line variants previously detected in our laboratory. Four out of 16 additionally identified variants were classified as disease-causing mutations according to the literature data, thus enabling us confirmation of clinical CF diagnosis in thee patients.

Conclusion: The NGS technology in combination with a well-characterized clinically relevant genomic variation database is a good alternative for a time consuming step-wise testing of genes with large allelic heterogeneity such as CFTR.