Targeted amplicon sequencing in genetic diagnostics of patients with cystic fibrosis

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Cystic fibrosis (CF), one of the most common autosomal recessive genetic disorders in Caucasians, is caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR). Almost 2000 variants in CFTR gene with variable clinical significance have been identified so far.

In this study we performed targeted re-sequencing of CFTR gene in 24 CF patients in which one or no mutations were identified after analysis of seven most common variants (c.1521_1523delCTT, c.489+1G>T, c.1624G>T, c.1652G>A, c.1657C>T, c.1585-1G>A, c.3909C>G) or after sequencing of the whole coding sequence of CFTR gene. Library pool was generated using multiplex PCR amplification (MASTR, Multiplicom) followed by sequencing on a MiSeq instrument (Illumina). Sequencing data were evaluated using the software Sequence Pilot (JSI Medical Systems). Identification of disease-relevant CFTR variants was assessed based on the CFTR database[1] and CFTR2 website[2].

The NGS sequencing data correctly confirmed 18 germline 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 three patients. The NGS technology in combination with a well-characterised clinically relevant genomic variation database is a good alternative for a time consuming stepwise testing of genes with large allelic heterogeneity such as CFTR.

1 www.genet.sickkids.on.ca/app
2 www.cftr2.org/