Direct diagnostics of hereditary diseases by nucleic acid analysis

DNA diagnostics helps to ascertain a clinical diagnosis. Once we know the genetic cause of a disease in a family we can follow a specific treatment or we can inspect other relatives and see if a person is in risk of developing such a disease and administer follow up strategy. DNA analysis may be performed on every biological sample. It depends on the stage of development (or age) of an individual which material will be withdrawn. DNA analysis is possible in pre-implantation period (one cell from embryo from in vitro fertilization), in prenatal period (chorionic villi sampling, amniocentesis, chordocentesis) and in post-natal period (any DNA containing material: blood, saliva, skin biopsy).

There are two basic strategies for DNA diagnosis

Direct: We focus directly on the gene in question, we search for DNA mutation that caused the dysfunction and lead to disease. ' *Indirect:*' We look at family pedigree and combine it with the value of specific polymorphism in tight linkage with the gene in question.

Direct diagnostics aim at methods capable of DNA mutations (sequence alterations) detection in genes that are causative for selected phenotype (disease). To examine DNA of an individual we first need to amplify (make thousands of copies) of a selected region of DNA. Method of amplification is Polymerase Chain Reaction (PCR). By this strategy we enrich the sample with DNA of interest and all downstream methods show physical (length) or chemical (presence of nucleotide at a specific position) characteristics of this amplified region. Phenotypes (diseases) that apply for direct DNA diagnosis must be monogenic (or oligogenic), where only one gene defect leads to a specific phenotype.

“One gene – one phenotype” enables to screen the coding parts of a single gene. Examples of such cases are: Cystic fibrosis – CFTR gene, PKU – PAH gene, Huntington disease – HTT. There are several methods that apply (PCR, RFLP, MLPA, NGS – discussed elsewhere), but they all aim at **coding parts, exon-intron boundaries, proper number of exons, sequence variations.**

A typical finding in direct DNA diagnosis is a causal mutation. For example, **PAH c.964 A>T, p.K322X** says, that due to a substitution of Adenine to Thymine at a coding position 964 there was a misincorporation of Stop codon (X) at the protein position 322 (instead of an aminoacid Lysin (K)). The final protein is shorter and cannot perform its regular function.

When a disease has variable genetic cause (deafness), regarding which gene is in question, one can apply panel NGS sequencing (massive parallel sequencing), where a group of genes is sequenced at once (typical panel consists of tens to hundreds of genes). If a gene is newly being linked to a selected phenotype, the aim is to incorporate in specific NGS panels and broaden the spectrum while increasing benefits and lowering costs.

When there is strong family support for inheritance pattern in a pedigree, but no known genes show mutation, one can apply Whole Genome Sequencing (WGS) of Whole Exome Sequencing (WES) and analyze all (if possible) family members. The results of WGS or WES (let say 20 thousands of genes) are compared with results of healthy family members and only gene defects that were shared by affected members and not present in healthy members are further evaluated. WGS has an advantage of screening the whole genome, whereas WES analyzes only coding parts of the genome. Research groups worldwide are more and more focusing on regulation sequences (enhancers, silencers, miRNA binding sites, 3´UTR and 5´UTR) that could have also a great impact on disease phenotype, but this DNA diagnostics are far from being routine.

In the Czech Republic, there are some common DNA mutations among families with Cystic fibrosis. The first step after isolation of DNA is the PCR amplification of selected regions of CFTR gene (regions that cover the most common DNA mutations). Final segments are analyzed for the presence/absence of the selected mutation. For example, **Delta F508 (c.1521_1523delCTT)** (the most common CFTR one codon deletion mutation – 67% of carriers) can be easily diagnosed by evaluation of the amplified fragment size; **c.1652G>A** (third most common, 3% of carriers) can be detected by restriction of the amplified fragment by an enzyme that recognizes this mutant allele, and evaluation of final fragment size. If all examined regions did not show mutation, analysis must be broadened to the whole gene (exons with adjacent intron parts) or there must be an inspection of the proper number of exons (evaluation of duplication or deletion of the whole gene or of his parts) by performing MLPA. Combination of both of these approaches can be achieved by NGS CFTR gene sequencing.

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