ABSTRACT

During the last years we have observed a rapid development of molecular genetic diagnostics (DNA diagnostics). New methods and technologies are rapidly being introduced and the spectrum of genetic services is gradually extended. Since germline genetic tests might have lifelong influence health and quality of patient's life, all efforts should aim at improvement of the overall quality of provided diagnostic services. An increasing number of laboratories replace their "in-house" developed techniques by the commercial diagnostic assays, but they often modify manufacturer's instructions. Therefore, it is necessary to validate and verify all methods and techniques before their implementation into routine DNA diagnostics.

In this thesis I have focused on evaluation and application of High Resolution Melting (HRM) in clinical diagnostic practice based on its comprehensive validation, according to the major international quality assurance standard ISO 15189. On the model of selected genes (*BRCA1, MTHFR, CFTR*) we have confirmed the high utility of HRM for mutation scanning of unknown variants, as well as genotyping of common variants. Concurrently, we have provided a list of methodical guidelines which could be applied for setting up HRM in other genetic laboratories and provided a diagnostic validation strategy for other DNA diagnostic techniques.

Furthermore, we have contributed to the higher quality of genetic services in the area of diagnostics of cystic fibrosis. This common life-threatening autosomal recessive disease is known for a substantial number of mutations in the *CFTR* gene and for its molecular heterogeneity based on the patient's ethnicity. Therefore, it is important to analyse mutation distribution and frequency of *CFTR* gene mutations among different populations. In this thesis, I have presented a comprehensive overview of CFTR mutations at Czech and Ukrainian CF patients, since identification of both causal mutations will support a clinical diagnosis, allow clinical prognosis, individually assess appropriate medical treatment, provide a reliable prenatal diagnostics and determine the risk for other family members. The high population detection rate will enable implementation of CF newborn screening, which helps to find CF patients before symptom occurrence. Such an early establishment of CF diagnosis and an immediate application of medical treatment favourably influence the overall clinical outcome and reduce the costs for treatment in this disease.

Human infertility is a serious medical and socio-economic issue since it currently affects approximately 15% of couples and this number is still increasing. The "male factor" in reproductive failure accounts for 50% of all cases, while many causes still remain unknown. Therefore, we performed a mutation analysis of protamine genes (*PRM1* and *PRM2*), as they play a crucial role in differentiation of spermatids. It was demonstrated that knockout (KO) of either protamine gene in mice results in male infertility due to an alteration in sperm chromatin assembly and nuclear integrity. These *Prm1* or *Prm2* haploinsufficient mice produce sperm exhibiting abnormal morphology, combined with reduced motility and are thus unable to fertilize an oocyte. We sequenced both genes in representative groups of German idiopathic infertile patients with distinct teratozoospermia and normal (resembling the phenotype of the KO mice) or reduced sperm concentration and in normozoospermic men as a control, in order to investigate the impact of protamine gene sequence variations on spermatogenesis. We have revealed a statistical significant association of ACC haplotype, formed by the three common SNPs of *PRM1/2* genes, and sperm concentration/count. Homozygous carriers of ACC haplotype had a twofold higher sperm concentration and count than men lacking this haplotype ($45x10^6$/mL x $24.2x10^6$/mL). Spermatozoa without the ACC haplotype might not be viable or might be subjected to negative selection. For the clinical impact of this finding and its implementation to the diagnostics it is necessary to confirm results by other studies on different cohorts and/or populations.