CHEK2 gene codes for serin/threonine kinase Chk2 (Checkpoint kinase 2). In response to genomic DNA damage, Chk2 phosphorylates its substrates (proteins Cdc25C, BRCA1 or p53), whose activation leads either to cell cycle arrest, DNA damage repair or induction of apoptosis. Germline mutations in CHEK2 gene increase risk of cancer development. Analysis of high risk breast cancer patients in Czech Republic reveals rare CHEK2 mutations (mainly missense) with yet unknown clinical significance.
This work focuses on functional impact of these variants and analysis of kinase activity of variant isoforms of Chk2 kinase. For this purpose, recombinant constructs were expressed in bacterial cells of E. coli. Enzymatic activity of Chk2 kinase isoforms in crude cell lysates was measured by the phosphorylation of Chk2 arteficial substrate spectrophotometrically. Results of in vitro kinase assay were correlated to the results of in silico prediction software.
The results show that from 15 analyzed mutations (together with one in frame deletion), kinase activity was abrogated in all variants affecting the kinase domain of Chk2, in concordance with in silico predictions. The same result has been found for a FHA domain variant p.R145Q. No significant changes in kinase activity were observed in case of two FHA domain variants (p.I157T a R181H) which coresponded to benign in silico prediction. While R181H results are in agreement with results of mutational analysis in risk populations, I157T has been considered to be a pathogenic variant that increase risk of the breast and colon cancer development. The results in other studied variants remained inconclusive and will require additional analysis to establish their significance.
The results indicate that functional analysis of variant CHEK2 kinase activity in vitro is the most appropriate for analysis ofvariants in Chk2 kinase domain in which it may help to identify clinically significant hereditary CHEK2 gene variants