Summary

The aim of the study was to search for the defects in the entire mitochondrial genome of dogs with malignant tumours of the mammary gland using the *Next Generation Sequencing* (NGS) method and bioinformatics tools and to assess their association with the neoplastic transformation process. From fourteen dogs with malignant tumours of the mammary gland, a tumour tissue and peripheral blood were collected. The histopathological evaluation of the tumours was performed in accordance with the current WHO (*World Health Organisation*) standards and the classification of malignancy (Goldschmidt i in., 2011). The isolated DNA was subjected to NGS sequencing and sequences with at least 100-fold overlap were obtained. The obtained sequences were subjected to bioinformatics analyses in order to determine the nature of the changes and their impact on the genes encoded in the mitochondrial DNA.

For the first time, polymorphisms, mutations and heteroplasmy were identified in the entire mitochondrial DNA genome of the dog (16,727 base pairs, 37 genes + the entire non-coding region). A total of 557 changes in mitochondrial DNA were identified in 13 dogs with mammary gland tumours of G1 malignancy grade and 47 changes in a dog with mammary gland tumour of G2 malignancy grade. Most mutations and heteroplasmy were detected in the VNTR (*variable number of tandem repeats*) area. Mutations in this region are believed to be responsible for the instability of the cancer cell genome. Changes occurred in 12 of the 13 protein-coding genes. In the vast majority, they were synonymous or non-synonymous with a low degree of harmfulness. The greatest number of polymorphisms was observed in the *COX1* gene, and the only protein gene in which no mutations and polymorphisms were observed was the *ATP8* gene, which proves its high genetic conservatism. Polymorphisms occurring in all samples occurred in the following genes: *tRNA-Leu (UUR)* (m.2678_2679insG*)*, *COX1* (m.5367C>T, m.5444T>C, m.6065A>G), *ATP6* (m.8368C>T), *COX3* (m.8807G>A), *ND4L* (m.9911_9912insTG), *ND5* (m.13299T>A) and non-coding region (m.15814C>T). It was shown that the effect of the 13594G>A polymorphism was a non-synonymous change of p.Gly606Glu in the ND5 protein and a synonymous change of p.Thr172= in the ND6 protein. The m.2683G>A polymorphism corresponded to the m.3243A>G position responsible for the MELAS (*mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes)* syndrome in humans and associated with many human cancers. The differences found at the molecular level in the D-loop area between two tumours of the same histopathological type occurring simultaneously in one dog may indicate that this area plays an important role in the process of malignant transformation. Mutations in the area of a variable number of tandem

repeats were mainly heteroplasmy and could contribute to the genetic destabilization of cancer cells.

Key words: dog, mtDNA, mutations, tumour