„X-chromosome inactivation in metabolic disorders and in healthy individuals”
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X-chromosome inactivation (XCI) is an epigenetic process involving the transcriptional silencing of one of the two X chromosomes in females during early embryogenesis. Thanks to this phenomenon, it is possible to balance the expression of genes located on the X chromosome in females (XX) and in males (XY). XCI is an inherently random phenomenon, meaning that the ratio of the number of cells with an inactive X chromosome inherited from the mother to the number of cells with an inactive X chromosome inherited from the father should be approximately 50:50. However, there are cases of significant deviations from this ratio. Studies to date have shown that skewed XCI is a quite common phenomenon, and its incidence increases with the age of females. The inactivation pattern depends on the type of tissue tested, it can vary in different tissues or organs, ranging from 50:50 to 80:20 in the same person. There are no indications that the degree of randomness of X-chromosome inactivation has any clinical significance in healthy females. On the other hand, in females who are carriers of the mutated gene located on X chromosome, clinical symptoms of the disease may be revealed if the normal allele is inactive in most of the cells.

This doctoral dissertation aimed to compare the X-chromosome inactivation pattern in the general female population in different tissues, to determine the effect of *in vitro* fertilization on the XCI phenomenon, and to better understand the X-chromosome inactivation process in humans. As part of this research, the XCI pattern was compared between readily available biological samples (saliva, buccal swab and blood) of females in different age groups. In order to elucidate the effect of *in vitro* fertilization on the process of X-chromosome inactivation in humans, the pattern of XCI in the saliva, buccal swab and blood of girls conceived *in vitro* and naturally was compared. To better understand the mechanism of the X-chromosome inactivation process in embryonic development, the XCI pattern in placental tissue and umbilical cord blood was also determined, and the expression profile of selected genes in samples with different XCI pattern was examined.

Another aim of this study was to analyze the X-chromosome inactivation pattern in heterozygous females with a family history of Fabry disease and to evaluate the impact of skewed X chromosome inactivation on the occurrence of Fabry disease symptoms in females.

The results obtained as part of the doctoral dissertation confirmed that skewed X-chromosome inactivation is quite common among females and increases with age. Skewed

X-chromosome inactivation was observed to increase with female age in saliva-derived cells but not in other tissues analyzed. It was found that the XCI pattern differed between tissues tested, while the XCI pattern determined in DNA isolated from saliva samples was more similar to the XCI pattern determined for blood than the pattern established for buccal swab. The presented studies showed no significant differences in the X-chromosome inactivation pattern in the tissues of *in vitro* and naturally conceived girls, indicating that assisted reproductive techniques are unlikely to interfere with the X-chromosome inactivation process. The results of the presented study showed that the XCI pattern differed between samples taken from the same placenta, which confirmed that the X-chromosome inactivation in extra-embryonic tissues in humans is a random phenomenon and not the result of imprinting, as is the case, for example, in mice. In addition, a different expression profile of several of the selected genes was observed in the placental tissues, in particular, in the placental tissue samples with skewed XCI >80:20 an increased level of gene expression was found for: *CD44*, *KDM6A*, *PHLDA2* and *ZRSR2*.

Analysis of the pattern of X-chromosome inactivation in female carriers of Fabry disease showed that skewed X-chromosome inactivation is not the main factor responsible for the manifestation of the disease in heterozygous females. It was observed that female carriers of Fabry disease presented a varied intensity of severity of symptoms - from mild to severe form of the disease, while their clinical manifestation did not correlate with the profile of X-chromosome inactivation. Among heterozygous females with a random XCI, symptoms of Fabry disease may vary in severity.