Abstract

In comparison to men, the number of X-linked genes is doubled in women as they have two chromosomes X while men are hemizygotes for X-linked genes. This imbalance is compensated by X inactivation (XCI) process, also known as primary X-inactivation, occurring in the early stage of embryogenesis. X inactivation is a random process and females are mosaics of two cell populations. The ratio of expressed alleles in women can be random (50:50) or skewed (≥80:20). The skewed X inactivation may occur due to selection when one of the alleles is preferentially inactivated (secondary X inactivation).

In this study XCI status in heterozygous females with various severity of phenotypic symptoms and traits in selected X linked inherited metabolic diseases is analysed, with the focus being Fabry disease - the deficiency of the enzyme alpha-galactosidase A encoded by GLA gene. Moreover, XCI in one family with X linked agammaglobulinemia is examined.

Mutant alleles and XCI status based on various loci, different methodical approaches and different tissues is subjected to examination. For the first time, the direct analysis of GLA gene transcript to detect the allele ratio was used alongside with the single-nucleotide polymorphisms in the IDS and LAMP genes for allele-specific expression (ASE) and the AR, RP2 and CNKSR2 loci for methyl-sensitive restriction analysis (MSRA). RNA samples for ASE methods were isolated from blood and DNA samples for MSRA were isolated from blood and buccal swabs. The reliability of probes according to the direct analysis of GLA gene transcript was tested and the tissue specificity in blood and buccal swabs was observed.

The high degree of correlation was detected between the probes. The study of tissue specificity revealed skewed X inactivation ratio (XIR) in 25 % of patients in blood samples while the buccal swab samples showed only one skewed XIR. There was a concordance detected between the activity of enzyme alpha-galactosidase A and the results of relative expression of GLA gene. The results of ASE and MSRA methods were compared to the clinical manifestations severity of Fabry disease and X-linked agammaglobulinemia. The XIR in blood and buccal swabs did not correspond to the phenotypic affection of patients. The more significant factor in Fabry disease are probably the type of mutation and patient's age. The XIR in X linked agammaglobulinemia is affected by secondary XCI.