Abstract

Skewed X chromosome inactivation has been often studied as a possible factor that influences manifestation of X-linked diseases in heterozygous women. Yet the association between phenotype and degree of skewing stays unclear for most disorders. Current works rely mostly on methods that are based on methyl-sensitive restriction while determining the X inactivation pattern and mainly the HUMARA assay which investigates the methylation profile in the *AR* gene. However those methods have some known disadvantages and therefore we are still seeking new methodical approaches.

We used DNA isolated from whole blood and in some cases also buccal swabs to asses X inactivation patterns in 54 women using methylation-based methods for loci *AR*, *CNKSR2* and *RP2*. Transcription-based assay was utilized to evaluate skewing of X inactivation in 32 of those women, whose samples were available for RNA extraction, using massive parallel sequencing and polymorphisms *LAMP2* c.156A>T, *IDS* c.438C>T and *ABCD1* c.1548G>A.

Partly thanks to almost no stuttering during PCR the *RP2* locus was the most informative in our study (71 % of women) and approximately the same number of women (69 %) were informative for the HUMARA assay. However when comparing the results of those two methods we determined difference greater than 10 % in several cases. Similarly the X inactivation patterns assessed with the transcript-based method (which was informative for 40 – 62 % of women in our group depending on individual polymorphism) were in most cases in a very good agreement with those obtained by methylation based assay although they also differed significantly in some cases. Therefore the agreement intervals were about 20 % wide for comparison of most of the methods we used. Based on determined coefficients of repeatability we assume that inaccuracy of measurement was the main cause of observed differences and it seems that the parallel sequencing method provides more reproducible results than assays based on methyl-sensitive restriction.

Based on our observation we consider the transcript quantifying method suitable for evaluation of X inactivation pattern in research and clinical use. However we also recommend using repeated measurements and combination of two or more methods to obtain the best results.

Keywords: chromosome X inactivation, skewed X inactivation, HUMARA, massive parallel sequencing