

NATURE AND MECHANISM OF GENOMIC IMPRINTING: STUDIES ON THE HUMAN IMPRINTED REGION ON CHROMOSOME 11p15.5

V. Bajaj, Ankita, Mahmood Ali and Arun Kumar

Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore-560012, India.

Aim: To identify imprinting status of genes and understand the nature and mechanism of genomic imprinting using genes from the human imprinted region on chromosome 11p15.5 as examples. **Methodology:** Two genes *SLC22A1LS* and *TAPAI* whose imprinting status was not known were selected. In order to determine allele specific expression pattern, PCR primer sets from the *SLC22A1LS* and *TAPAI* genes were used to find heterozygosity in DNA samples from 17 spontaneous abortuses using PCR-SSCP and DNA sequence analyses. cDNA samples from different tissues of spontaneous abortuses showing heterozygosity were subjected to RT-PCR-SSCP analysis to determine the allele specific expression pattern. To find the nature and mechanism of genomic imprinting, four genes *SC22A1LS*, *TSSC3*, *ZNF215* and *SLC22A18* were selected. As imprinted genes are known to show differentially methylated regions (DMRs) in their promoters and acetylation and methylation of lysine residues in H3 and H4 histone proteins, deletion constructs of the putative promoter regions of these genes were made in the pGL3 basic promoter less vector. The constructs were transfected in human cell lines for luciferase assay. The promoter regions were then screened for polymorphisms to differentiate paternal and maternal alleles by sequencing the regions in 17 fetuses. DMRs will be identified using sodium bisulfite mutagenesis. Chromatin immunoprecipitation (ChIP) assays will be used to study the acetylation and methylation of lysines in histone proteins. **Results and conclusions:** PCR-SSCP analysis revealed heterozygosity in the *SLC22A1LS* gene in two of the 17 abortuses examined. RT-PCR-SSCP analysis showed that this gene is paternally imprinted in five fetal tissues. Studies are in progress to isolate the promoter of this gene. *TAPAI* showed biallelic expression. We have also isolated the promoters of two other imprinted genes, *SLC22A18* and *TSSC3*. We are in the process of identifying a) the DMRs in the promoters of *SLC22A18* and *TSSC3*, b) promoters and DMRs of *SLC22A1LS* and *ZNF215*, and c) acetylation and methylation of lysine residues in these genes. (This work is financially supported by a DST grant to AK).