Abstract

Introduction: Glycoproteins of the major histocompatibility complex (MHC) are an irreplaceable part of immune response regulation and immune homeostasis maintenance. The regulation of the expression plays an important role in adaptive immune response. Recently, DNA methylation in regulatory areas, crucial for DNA availability to transcription factors, is one of the most researched mechanisms of this type of regulation. The DNA methylation is, among others, related to the aging processes. Increased predisposition age-related immunosenescence in higher age could result from the changes in methylation status of regulatory areas of MHC class II genes.

Aims: The aim of this thesis is to analyze the methylation status of regulatory areas of *DQB1* gene and to compare the differences between generations and specific alleles. The differences in the levels of DQB1 gene mRNA transcription between generations and specific alleles is also compared.

Methods: Both DNA and RNA were isolated from blood samples obtained from donors of three different age groups. DNA was genotypized and modified by bisulfite conversion. The regulatory areas of DQB1 genes were then amplified and subcloned into bacteria. The positive clones were selected and subjected to DNA methylation analysis. RNA was reverse transcribed into cDNA and its relative level of expression was determined by quantitative PCR.

Results: The intergeneration analysis of intron 1 showed statistically significant difference in methylation of allele 06:02 which apears to be methylated more in chidren compared to mid-age individuals. Interallelic comparison inside separate groups was statistically proven for intron 1 in children. The allele 05:01 is methylated more prominently than allele 06:03. Intron 2 of allele 05:03 is methylated less than in allele 05:01. The mid-age group exhibited lesser methylation in allele 06:02 intron compared to alleles 06:03 and 03:01,similar trend was observed in senior group for allele 06:02 intron 1 compared to allele 05:01. The comparison of specific methylation sites inside given allele was statistically confirmed for sites 1298, 1621, 1661 and 2248 in allele 06:02. This allele appears to be methylated more in these sites in children,compared to mid-age individuals. Compared both to mid-age and senior individuals, this allele also exhibits stronger methylation in children in 1706 site. Closer examination of allele 05:01 discovered stronger methylation in children in sites 1511,1517, 1563 and 1575, compared to senior group. The relative level of

expression analysis showed higher expression of allele 06:02 compared to alleles 02:02 and 03:01 and lower level of methylation in allele 06:02 for seniors individuals in comparison to mid-age group.

Keywords: HLA class II, HLA DQB1, epigenetics, expression regulation, DNA methylation, aging