Abstract

Background: The major histocompatibility complex (MHC) molecules play an important role in the immune response regulation and in the maintenance of the immune homeostasis. Regulation of their expression is therefore a key factor influencing the adaptive immune response. DNA methylation of gene regulatory regions is one of the mechanisms of gene expression control that affects the accessibility of DNA to transcription factors. Ageing is connected with changes in DNA methylation and increased predisposition to autoimmune diseases in older age could be associated with changes in MHC class II genes methylation.

Aims: The aim of this diploma thesis is to analyze the methylation profile of *DQA1* and *DQB1* genes regulatory regions and to compare its differences between the generations and between individual alleles. The next aim is to compare *DQA1* mRNA expression between the generations and between single alleles.

Methods: DNA and RNA were isolated from blood of three age group donors. DNA was converted by the bisulfite treatment and regulatory regions of HLA class II genes were amplified and cloned into bacteria. Positive clones were sequenced and then analyzed. RNA was reverse transcribed and its expression level was determined by real-time PCR.

Results: Statistically significant differences were found by intergenerational comparison of promoter region methylation of *DQA1* gene alleles. QAP allele 1.1 was methylated more in students than in generation of seniors at positions -562 and -540 from the transcription start. Other significant difference was found by comparison of individual alleles within the students group, where QAP alleles 1.3 and 4.1A were less methylated than the other alleles. The same outcome was found for QAP allele 1.3 in seniors group. The reason why similar results were not found in the group of children may be the small number of samples. Statistically significant difference was also found by analysis of regulatory region of the *DQB1* gene. Allele *DQB1*0602* was methylated less than allele *DQB1*0501* at position 2235 nucleotides from the transcription start.

Key words: HLA class II, HLA *DQA1*, HLA *DQB1*, epigenetics, DNA methylation, ageing