

Abstract

Background: Type 1 diabetes (T1D) is a multifactorial autoimmune disease. Its incidence in Europe is continuously rising. The highest T1D risk is associated with HLA (human leukocyte antigen) class II genes. HLA class II molecules play a key role in regulation of immune response. They contribute to the selection of T cell repertoire by presenting antigenic peptides to the CD4⁺ T lymphocytes. HLA class II expression is controlled by regulatory module that is situated 150 - 300 base pairs upstream of the transcription-initiation site in all HLA class II genes. Polymorphisms in this region are linked to some autoimmune diseases. There were identified several promoter alleles (named QAP) in the *HLA DQA1* gene promoter region. Most of the polymorphisms appear to be conserved within haplotype. Individual QAP alleles may have a different promoter strength by which they influence expression of *HLA DQA1* gene alleles. Promoter strength can be modulated by DNA methylation.

Aims: Our aim was to define methylation profile of *HLA DQA1* promoters and determine the mRNA expression of individual alleles of *HLA DQA1* gene in T1D patients. The mRNA expression level of *HLA DQA1* gene alleles was determined using quantitative PCR.

Methods: 30 diabetic patients (age range 21 to 76 years), were included in this pilot study. The genotyping of *HLA DRB1*, *HLA DQA1* and *HLA DQB1* was performed using PCR with sequence specific primers. Genomic DNA was treated with sodium bisulfite, promoter region of *HLA DQA1* gene was amplified and cloned into the *E. coli*, strain DH5 α . Positive transformants were selected on agar plates containing ampicillin X-Gal, and IPTG. Positive transformation were confirmed by colony PCR. Sequencing of individual clones was performed.

Results: We found statistically significant differences in total QAP methylation and in methylation at nucleotide position -311. The most significant result in total QAP methylation was found between 2.1 and 1.3 alleles (P = 0.0280). While 2.1 QAP allele was completely methylated at position -311, 1.4 allele was not methylated at all (P = 0.0064, CI (95%) = 0.00009178 – 0.3116). The mRNA expression level of *HLA DQA1**03 was higher than the expression of other *HLA DQA1* alleles.

Key words: Type 1 diabetes mellitus, HLA class II, HLA DQA1, promoter, polymorphism, DNA methylation.