

Abstract

Introduction: Celiac disease is a multifactorial autoimmune disease that is caused by a response to gluten and related proteins in genetically susceptible individuals. Genetic studies have demonstrated a high association of celiac disease with HLA genes (human leukocyte antigens) II. Class. Approximately 90-95% of the patients have a HLA-DQ2 haplotype (DQA1*05/DQB1*02), the remaining 5-10% are carriers of HLA-DQ8 (DQA1*03/DQB1*03) or inherited only one allele of HLA-DQ2 genotype. Approximately 30% of the healthy population has this haplotype and only 1% develops celiac disease. If 30% of healthy individuals have this haplotype and do not develop celiac disease, this may be affected by a different level of expression in healthy and diseased individuals.

Aims: The aim was to design and test a primer and probe system for QPCR that will amplify all selected HLA-DQA1 and HLA-DQB1 candidate genes at risk for celiac disease and use this system to measure the relative level of expression of risk genes in healthy donors and patients with celiac disease.

Methods: The study included 10 patients with recent celiac disease and 15 healthy donors who were selected from the database of the Institute of Medical Genetics of the 3.LF UK based on their genotypes HLA-DQA1 and HLA-DQB1. Patients were first detected their HLA II. Class genotype by sequence-specific primers. From the collected blood, mRNA was isolated and subsequently transcribed into the cDNA. Using QPCR, the relative level of expression of selected HLA-DQ risk genes was measured.

Conclusion: A primer and probe system for QPCR has been designed and optimized to measure the relative expression of selected candidate celiac genes. The system was designed for HLA-DQA1 alleles (DQA1 * 05, DQA1 * 02: 01, DQA1 * 03: 01) and HLA-DQB1 alleles (DQB1 * 02, DQB1 * 03: 02). Additionally, the system was designed to the HLA-DQA1 intron domain, which makes it possible to determine the degree of contamination of the gDNA in the sample. To test proposed systems, the expression of interest alleles in the pilot set of healthy donors and patients with celiac disease was measured. The aim of the work was not to draw conclusions from the results of the individual haplotypes, but the data obtained suggest that a different expression of the risky alleles in untreated celiac disease can be expected and that it will also be interesting to focus on the variability of expression within the groups.

Key words: celiac disease, HLA II. Class, HLA-DQA1, HLA-DQB1, polymorphism, genetic expression