P622

A New Method for Detecting Polymorphisms in the TPMT Gene Based on Real Time PCR

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The TPMT (Thiopurine S-methyltransferase) enzyme is responsible for a major inactivation route of thiopurines (azathioprine, 6-mercaptopurine and 6-thioguanine), drugs which are used to treat acute lymphoblastic leukemia (ALL), various autoimmune or chronic inflammatory conditions, such as inflammatory bowel disease (IBD; Crohn's disease CD, ulcerative colitis UC), rheumatic arthritis, psoriasis and their immunosuppressive action prevents the rejection of transplant organs. About 10-28% of patients experience adverse drug reactions (ADRs) related to the treatment with thiopurines. The most serious reaction is myelosupression, typically manifested as leucopenia, which occurs in about 2-5% of patients. Other ADRs that accompany thiopurine therapy are pancreatitis, hepatotoxicity, allergic reactions, digestive intolerance, arthralgia, febrile conditions and rash.

Many clinical studies confirmed the correlation between the occurrence of leucopenia in patients treated for azathioprine and the occurrence of variant alleles TPMT*3A (presence of substitution polymorphisms 460G>A [rs1800460] and 719A>G [rs1142345]), TPMT*3C (719A>G), and TPMT*2 (238G>C [rs100462]). These three alleles are present in 80-95% of individuals with intermediate or low enzyme activity.

TPMT genotyping of patients treated with azathioprine is an example of the use of pharmacogenetics in clinical practice. This examination has become a part of routine laboratory testing before the initiation of the azathioprine therapy, and helps physicians identify patients at increased risk of side effects during the treatment. Based on the observed TPMT genotype, it is possible to choose individual drug doses required to achieve the desired therapeutic effect.

There are many methods to determine the TPMT genotype but most of them are methodically demanding and time consuming. It requires the use of three independent PCR reactions, restriction cleavage and electrophoresis to evaluate results.

We have designed a methodology based on Real Time PCR using TaqMan dual-labeled probes. This methodology allows genotyping of all three polymorphisms under the same reaction conditions and in parallel PCR reactions, i.e. at the same time. This adjustment maximally simplifies the procedure, minimizes individual error, reduces the time required for genotyping and is therefore suitable for the routine use in clinical practice. Specificity and reproducibility of this methodology has been verified on a set of 188 patients diagnosed with IBD with known TPMT genotypes. The results obtained by this new method were in 100% agreement with genotyping performed by other methodologies. There is currently no unified methodology for the determining of these variant alleles. We believe that our upcoming kit for the determining of TPMT genotype, based on this methodology, will find use in clinical practice due to its indisputable advantages.

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