DETERMINATION OF GENETIC POLYMORPHISMS AFFECTING METABOLISM OF THIOPURINES

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Objetive

Treatment with thiopurines can cause toxicity if the patient is a carrier of certain polymorphisms in the *NUDT15* gene, which encodes some enzymes involved in the metabolism of this drug. A technique has been optimized to identify mutations that abrogate or reduce the activity of NUDT15.

Materials

Determination polymorphisms were performed by PCR and subsequent sequencing. PCR conditions were:

30” at 98°C; 40 cycles x (10” at 98°C; 10” at 55°C; 10” at 72°C); 2’ at 72°C

The design and specificity of the primers were performed with the Gene Runner® and Primer-Blast programs, respectively. Reading the chromatograms it was carried out with the program *Mega*®. DNA extraction was performed from a drop of blood deposited on paper Whatman™ 9031 (1).

Results

Table 1 shows the sequences of the primers used for amplification of genomic DNA fragment and the "internal" used in the sequencing thereof. Each pair of oligonucleotides allows us to identify more than one polymorphism. The yield of the reaction was optimum, allowing its subsequent sequencing.

Discussion

With our work we wanted to show a simple and economical method, accessible to any laboratory with basic equipment in molecular biology, which allows us to detect previously in patients mutations that adversely affect the metabolism of thiopurine.

Variant ID	Sequences	Tm	Amplicon size (bp)
Rs116855232 Rs147390019	F: GCATCACTATGAGTTTATTAGTAGC R: CACCAGATGGTTCAGATCTTC S: CACTATGAGTTTATTAGTAGCAAG	60,7° 60,5° 56,9°	253
Rs186364861 Rs554405994	F: ACGCATTACGCACCGC R: GCTCACCCGAACTCCAGAT S: CGCTATGACGGCCAG	62,1° 63,2° 55,7°	324 or 318 (indel)

Table 1. *F: Forward amplification primer; R: Reverse amplification primer; S: Sequencing primer; Tm: melting temperature; bp: base pair*

Conclusion

Genotyping *NUDT15* allows individualizing treatment doses, as a carrier for any of these mutations. Regions encompassing the four polymorphisms were amplified using only two primer pairs. The direct costs associated with the determination of four markers was 27 € (6,75 € each polymorphism). Future studies will determine the initial dose of the drug.

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(1) Ramos-Díaz R, et al. Validation of a fast and low-cost alkaline lysis method for gDNA extraction in a pharmacogenetic context. *Cancer Chemother Pharmacol*. 2015;75(5):1095-8.