Supplemental Digital Content

Methods

DPYD gene variant analysis by Sanger sequencing

PCR reactions were performed in a 20 µL reaction containing 30-50 ng of DNA, 2 µL of 10x Taq reaction buffer, 1.5 µL of MgCl2 (1.875 mM), 1 µL of deoxynucleoside triphosphate mix (dNTP) (250 µM dTTP, 250 µM dATP, 250 µM dGTP, 250 µM dCTP,), 0.3 pmol/µL of primer forward and reverse (Supplementary Table 1) and 0.75 units of Taq DNA polymerase (Fermentas, Thermo Fisher Scientific). After a 95 ºC pre-incubation step for 10 min, PCR was performed in a total of 35 cycles using the following conditions: 95 ºC denaturation for 45 s, annealing at 58 ºC for 45 s, and extension at 72 ºC during 45 s, followed by 10 min of final extension at 72 ºC. Sequencing analysis was performed on a 3500 Genetic Analyzer using Big Dye Terminator Chemistry (Thermo Fisher Scientific), using M13 universal primers and according to the manufacturer's recommendations.

DPYD gene variant analysis by KASP assay methodology

KASP assay primers were designed using the Primer-BLAST tool [1] and are described in supplementary table 2. KASP genotyping reactions were carried out in a total volume of 8 µL containing 1 µL of sample DNA (20 ng/µL), 3.89 µL of 2X KASP Master mix, 0.11 µL of Custom KASP Assay and 3 µL of water. KASP reactions were performed on a Roche LightCycler 480 Real-Time PCR System (Roche Diagnostics International AG, Rotkreuz, Switzerland) using the endpoint genotyping workflow. To allow for background correction of fluorescent values during endpoint genotyping analysis, a pre-read was performed at 37°C for 5 sec. PCR amplification was performed using the following conditions: 94°C for 15 min, then 10 cycles of 94°C for 20 sec, 64°C for 1 min

(dropping by 0.8 °C per cycle), then 36 cycles of 94°C for 20 sec, and 56°C for 1 min. Fluorescence quantification was performed at 37°C for 5 sec. Endpoint genotyping analysis was carried out using the LightCycler 480 Software 1.5.0 (Roche), using the background correction option.

References

1. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics* 2012;**13**:134.

Tables

Supplementary table 1 PCR amplification primers used for the analysis of the four *DPYD* variants by Sanger sequencing.

Variant	Primer sequence
c.1129-5923C>G	F: TGTGCAATCTAAAAGTGAGTTGAC R: ATCAAGCAAACATGCCAACCT
c.1679T>G	F: AAGCCTGAACTACCCCTCTTTTAC R: CTTCTTCCATGGGACAGAAAGGAA
c.1905+1G>A	F: GCTTTTCTTTGTCAAAAGGAGAC R: CCAACTTATGCCAATTCTCTTG
c.2846A>T	F: GCTTGCTAAGTAATTCAGTGGCT R: AGAAGAGCAATATTTGGCACCAC

Forward (F) and reverse (R) primers were 5' tailed with a M13 universal sequence (TGTAAAACGACGGCCAGT and CAGGAAACAGCTATGACC for forward and reverse primers, respectively). *DPYD* variants are described using the reference sequences NG_008807.2 and NM_000110.3.

Supplementary table 2 KASP assay primers used for genotyping the four *DPYD* variants.

Variant	Primer sequence
c.1129-5923C>G	Allele C_F: TGAATATGGAGGTGAAAATCAAAGC Allele G_F: TGAATATGGAGGTGAAAATCAAAGG Common_R: AGTAAGCAGCTATAAGGGTCCA
c.1679T>G	Allele T_R: CATCCAGCTTCAAAAGCTCTTCGAA Allele G_R: CATCCAGCTTCAAAAGCTCTTCGAC Common_F: ACACTCCTATTGATCTGGTGGACA
c.1905+1G>A	Allele G_R: CTCTTGTTTTAGATGTTAAATCACACTTAC Allele A_R: CTCTTGTTTTAGATGTTAAATCACACTTAT Common_F: TTGAGCTCATCAGTGAGAAAACG
c.2846A>T	Allele A_F: AGAGCAAGTTGTGGCTATGATTGA Allele T_F: TAGAGCAAGTTGTGGCTATGATTGT Common_R: GCATTCTAATTCCAGCAGGATTCTT

The KASP primer assay is a combination of two allele□specific primers (one for each variant allele) and one common primer, designed in forward (F) or reverse (R) direction. The allele specific primers contain a unique unlabeled tail sequence at the 5' end that is complementary to two 5’ labeled primers, one with FAM and other with VIC, that are present in the KASP reaction mix. *DPYD* variants are described using the reference sequences NG_008807.2 and NM_000110.3.